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(54) Title: POLYPEPTIDES WITH SPECIFICITY FOR NEOPLASIAS, KIT, AND DIAGNOSTIC, VACCINATION, AND THERAPEUTIC METHODS

(57) Abstract

A polypeptide consists of a variable region of the light or heavy chains of an antibody of a species selectively binding the human mammary fat globule (HMFG) antigen, and an antigen found on the surface or the cytoplasm of tumor cells such as carcinoma cells or that is released by the cells, combinations thereof operatively linked to one another, and mixtures thereof. The polypeptide may be glycosylated. A hybrid polypeptide comprises the polypeptide of the invention and an effector agent, and both polypeptides may be present as a composition with a non-proteolytic carrier as well as with a pharmaceutically acceptable carrier. Diagnostic kits comprises the polypeptide or hybrid polypeptide of the invention and other components such as anti-tumor antibody, and anti-constant region immunoglobulin, protein G or A, a solid support, and instructions for their use. Tumors may be imaged and/or diagnosed in vivo by administering the radiolabeled polypeptide of this invention and detecting any localized labeled polypeptide, and in vitro by contacting a biological sample with the hybrid polypeptide of the invention to form a complex with neoplastic antigen present in the sample, and detecting any complex formed. The growth or the size of a primary or metastasized tumor may be therapeutically inhibited or reduced by administering the polypeptide or hybrid polypeptide of the invention. A polynucleotide comprises an oligonucleotide encoding the polypeptide or hybrid polypeptide described above, hybrid vectors carry the polydeoxyribonucleotide(s) of the invention, and host cells are transfected with the hybrid vectors. Te polypeptide or hybrid polypeptide may be obtained by cloning one or more of the polydeoxyribonucleotide(s) of this invention. An anti-idiotype polypeptide comprises polyclonal antibodies raised against the polypeptide of this invention; monoclonal antibodies thereof; Fab, Fab', $(Fab')_2$, and variable region fragments thereof; an oligopeptide comprising the amino acid sequence APDTRPA or tandem repeats thereof, combinations thereof and mixtures thereof. The hybrid polypeptide may also contain an effector agent and be provided as an anti-neoplastic vaccine in a vaccination kit. The serum concentration of a circulating polypeptide that binds to an antigen present on the surface or in the cytoplasm of tumor cells or that are released by the cells may be lowered by administering the anti-idiotype polypeptide of the invention to accelerate the clearance of the polypeptide.

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POLYPEPTIDES WITH SPECIFICITY FOR NEOPLASIAS, KIT, AND DIAGNOSTIC, VACCINATION, AND THERAPEUTIC METHODS

Field of the invention

This invention relates to the diagnosis, immunization, and therapy of neoplastic tumors of a subject of a first species, particularly carcinomas, by means of specifically targeted polypeptides comprising amino acid sequences encompassing, for instance, the variable (F_v) region of anti-tumor antibodies of a second species, among others. This invention provides the polypeptides as a single amino acid chain or as paired chains having the specificity of an antibody's F_v regions of the light or heavy chains of the second species, either by themselves or bound to other polymers such as synthetic polymers, e.g., oligopeptides, resulting in chimeric antibodies, e.g., human/non-human chimeric antibodies, and other polymeric constructs. The present polypeptides elicit a lesser immunological response in the subject treated, e.g. humans, than the complete sequence of the heterologous non-human antibody. Also provided herein are anti-idiotype antibodies and analogues, hybridomas, polynucleotides encoding the polypeptides of the invention, hybrid vectors, and transfected hosts expressing the polypeptides of this invention. The polypeptides of the invention are useful in the in vivo and in vitro diagnosis of neoplasias localized in different organs as well as in their in vivo therapy. The present polypeptides and anti-idiotype antibodies are also suitable for immunizing humans against neoplasias. The polynucleotide sequences, hybrid vectors and transfected hosts are useful for preparing the polypeptides described herein.

Description of the Background

Carcinomas result from the carcinogenic transformation of cells of different epithelia. Two of the most damaging characteristics of carcinomas are their uncontrolled growth and their ability to create metastases in distant sites of the host, particularly a human host. It is usually these distant metastases that cause serious consequences to the host, since frequently the primary carcinoma may be, in most cases, removed by surgery. The treatment of metastatic carcinomas, that are seldom removable, depends on irradiation therapy and systemic therapies of different natures. The systemic therapies currently include, but not fully comprise, chemotherapy, different immunity-boosting medicines and procedures, hyperthermia and systemic monoclonal antibody treatment. The latter can be labeled with radioactive elements, immunotoxins and chemotherapeutic drugs.

Radioactively labeled monoclonal antibodies were initially used with success in lymphomas and leukemia, and recently in some carcinomas. The concept underlying the use of labeled antibodies is that the labeled antibody will specifically seek and bind to the carcinoma and, the radioactive element, through its decay, will irradiate the tumor in situ. Since radioactive rays travel some distance in tumors it is not necessary that every carcinoma cell bind the labeled antibody. The specificity of the monoclonal antibodies will permit a selective treatment of the tumor while avoiding the irradiation of innocent by-stander normal tissues, that could be dose limiting. Chemotherapy produces serious toxic effects on normal tissues, making the chemotherapy of carcinomas less than desirable, and the use of radiolabeled monoclonal antibodies a valid alternative.

Non-human antibodies raised against human epitopes have been used for the diagnosis and therapy of carcinomas as is known in the art. Also known are the methods for preparing both polyclonal and monoclonal antibodies. Examples of the latter are BrE-2, BrE-3 and KC-4 (e.g., US patent Nos. 5,077,220; 5,075,219 and 4,708,930..

The KC-4 murine monoclonal antibody is specific to a unique antigenic determinant, the "antigen", and selectively binds strongly to neoplastic carcinoma cells and not to normal human tissue (U.S. Patent No. 4,708,930 to Coulter). The antigen appears in two forms in carcinoma cells, only the smaller of these forms being expressed in the cell membrane. The larger form appears only in the cytoplasm and has an approximate 490 Kdalton molecular weight (range of 480,000-510,000). The second form occurs at a higher density of expression, is found both in the cytoplasm and the membrane of carcinoma cells and has an approximate 438 Kdalton molecular weight (range of 390,000-450,000) as determined by gel electrophoresis with marker proteins of known molecular weights. Labeled KC-4 was applied to the diagnosis and medical treatment of various carcinomas, particularly adenocarcinoma and squamous cell carcinoma regardless of the human organ site of origin.

The BrE-3 antibody (Peterson et al., Hybridoma 9:221 (1990); US patent No. 5,075,219) was shown to bind to the tandem repeat of the polypeptide core of human breast epithelial mucin. When the mucin is deglycosylated, the presence of more tandem repeat epitopes is exposed and the binding of the antibody increases. Thus, antibodies such as BrE-3 bind preferentially to neoplastic carcinoma tumors because these express an unglycosylated form of the breast epithelial mucin that is not expressed in normal epithelial tissue. This preferential binding combined with an observed low concentration of epitope for these antibodies in the circulation of carcinoma patients, such as breast cancer

patients, makes antibodies having specificity for a mucin epitope highly effective for carcinoma radioimmunotherapy. A ^{90}Y-BrE-3 radioimmunoconjugate proved highly effective against human breast carcinomas transplanted into nude mice. Human clinical studies showed the ^{90}Y-BrE-3 radioimmunoconjugate to considerably reduce the size of breast tumor metastases without any immediate toxic side effects. Moreover, an ^{111}In-BrE-3 radioimmunoconjugate was successfully used for imaging 15 breast cancer patients, providing excellent tumor targeting in 13 out of 15 of the patients. Out of all the breast tumor metastases occurring in another study, 86% were detected by ^{111}In-BrE-3. Unfortunately, 2 to 3 weeks after treatment, the patients developed a strong human anti-mouse antibody (HAMA) response that prevented further administration of the radioimmunoconjugate. The HAMA response, which is observed for numerous murine monoclonal antibodies, precludes any long-term administration of murine antibodies to human patients. Similarly, other heterologous antibodies, when administered to humans, elicited similar antibody responses. The anti-heterologous human response is, thus, a substantial limiting factor hindering the successful use of heterologous monoclonal antibodies as therapeutic agents, which could, otherwise, specifically anhilate breast carcinomas, causing little or no damage to normal tissue and having no other toxic effects.

Chimeric antibodies are direct fusions between variable domains of one species and constant domains of another. Mouse/human chimeric antibodies prepared from other types of B cells binding to other types of antigenic determinants have been shown to be less immunogenic in humans than whole mouse antibodies.

Accordingly, there is still need for a product of high affinity and/or specificity for carcinoma antigens suitable for the detection and therapy of carcinomas which elicits a lesser antibody response than whole non-human antibodies.

SUMMARY OF THE INVENTION

This invention relates to a polypeptide which selectively binds to an antigen found on the surface or in the cytoplasm of cells of tumors such as carcinomas or that are released by the cells, the polypeptide consisting essentially of at least one variable region of the light or heavy chains of an antibody selectively binding the human mammary fat globule (HMFG) antigen and to antigen found on the surface or the cytoplasm of tumor cells such as carcinomas or that are released by the cells, combinations thereof wherein each polypeptide is operatively linked to at least one other polypeptide, or mixtures

thereof. The polypeptide may be glycosylated. This invention also encompasses a hybrid polypeptide comprising at least one polypeptide described above and at least one effector agent operatively linked thereto, combinations thereof, and mixtures thereof. The polypeptide and hybrid polypeptide are also provided as a composition comprising the anti-tumor polypeptide or hybrid polypeptide of this invention and a non-proteolytic carrier, such as a pharmaceutically-acceptable carrier. The present polypeptide or hybrid polypeptide may be prepared by a method comprising cloning the polydeoxyribonucleotide of this invention into a vector to form a hybrid vector; transfecting a host cell with the hybrid vector and allowing the expression of the anti-tumor polypeptide or hybrid polypeptide; and isolating the anti-tumor polypeptide or hybrid polypeptide or mixtures thereof. Also provided are hybridoma cells expressing the polypeptide of hybrid polypeptide of the invention, such as those having the ATCC Accession Nos. HB 11200 and HB 11201.

Stil part of this invention are diagnostic kits for neoplasias. One kit comprises, in separate containers, the anti-tumor polypeptide or hybrid polypeptide of this invention, anti-tumor antibody, and immunoglobulin, protein G or protein A, a solid support, and instructions for its use. The other kit comprises the hybrid polypeptide of the invention, wherein the effector agent is the constant region of a heterologous antibody or fragment thereof that binds immunoglobulins, protein G or protein A, a solid support having operatively linked thereto an antigen selectively binding the anti-tumor hybrid polypeptide and the antibody, and instructions for its use.

The polypeptide and hybrid protein of this invention may be applied to the in vivo imaging and/or diagnosing of a tumor such as a carcinoma by administering, to a subject suspected of being afflicted with a primary or metastasized tumor, the anti-tumor polypeptide or hybrid polypeptide of this invention, e.g. in radiolabeled form, in an amount effective to reach the tumor cells and bind thereto, and detecting any localized labeled polypeptide or hybrid polypeptide. A tumor may also be diagnosed in vitro by contacting a biological sample obtained from a subject with the anti-tumor polypeptide or hybrid polypeptide of this invention to form a polypeptide-antigen complex or a hybrid polypeptide-antigen complex with any tumor cell antigen present in the sample, and detecting any complex formed. The diagnosis may also be accomplished in vitro by contacting a biological sample with a known amount of the hybrid polypeptide of the invention having a constant region or fragment thereof and an antibody, in the presence of an antigen molecule that specifically binds the polypeptide being operatively linked to a solid support, to form a polypeptide-antigen molecule complex on the solid support and a polypeptide-sample antigen

complex with any neoplastic cell antigen present in the sample, detecting any complex formed between the polypeptide and the solid supported neoplastic cell antigen, and comparing the result with a control conducted in the absence of the sample.

The polypeptide and hybrid polypeptide of this invention are also useful for inhibiting the growth or reducing the size of a primary or metastasized tumor, for purging neoplastic cells from a biological fluid and for histochemical testing of tissues for the presence of neoplastic cells.

Also provided herein is a composition containing an oligonucleotide encoding the anti-tumor polypeptide and/or hybrid polypeptide described above, a hybrid vector carrying the polydeoxyribonucleotide of the invention operatively linked thereto, and a transfected host cell, carrying the hybrid vector.

Also disclosed herein is an anti-idiotype polypeptide comprising polyclonal antibodies raised against the polypeptide of this invention, monoclonal antibodies thereof, fragments thereof selected from the group consisting of Fab, Fab', $(Fab')_2$, and variable regions, an oligopeptide comprising the amino acid sequence APDTRPA or tandem repeats thereof, combinations thereof wherein each oligopeptide, antibody or fragment thereof is operatively linked to at least one other oligopeptide, antibody or fragment thereof, and mixtures thereof, and optionally an effector agent operatively linked to the anti-idiotype polypeptide. The anti-idiotype polypeptide of the invention may be used as an anti-tumor vaccine for the prevention and/or therapeutic treatment of or vaccination against tumors such as carcinomas, e.g. as a composition also comprising a pharmaceutically-acceptable diluent or carrier. An anti-tumor vaccination kit may suitable comprise the vaccine of this invention and a diluent, in separate sterile containers, and instructions for its use.

The serum concentration of a circulating polypeptide that cross-reacts with the polypeptide of the invention may be lowered by administering to a subject the anti-idiotype polypeptide of this invention in an amount effective to bind the circulating polypeptide and thereby accelerate its clearance.

Other objects, advantages and features of the present invention will become apparent to those skilled in the art from the following discussion.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

This invention arose from a desire by the inventors to improve on antibody technology suitable for use in diagnostic, vaccine and therapeutic applications. The monoclonal antibodies obtained up the present time have been prepared by fusing immortalized cell lines with B-cells of non-human origin such as murine, rat, rabbit, goat, and the like. Many of these hybridomas can produce

monoclonal antibodies that have desirable binding properties such as high affinity and/or specificity for human carcinoma antigens, and are also produced in large amounts. However, in general, non-human antibodies may only be administered once to humans due to the detrimental effects they produce. For example, the repeated administration of mouse antibodies to a human subject elicits a strong human anti-mouse antibody (HAMA) response, which precludes their further utilization as therapeutic agents in humans. These non-human antibodies initiate an immediate adverse reaction in the patient and are, thus, rendered ineffective as therapeutic agents. On the other hand. human monoclonal hybridoma cell lines have not been very stable and have, therefore, not been suitable for the large scale, repeated production of monoclonal antibodies.

Accordingly, the present inventors have undertaken the preparation of anti-tumor heterologous, e.g. human and non-human, variable regions and hybrid polypeptides comprising the heterologous variable regions to lower or even circumvent the endogenous antibody response to heterologous antibodies. The present invention utilizes the Fv regions of light and/or heavy chains of heterologous antibodies, such as those of mouse, human, rat, rabbit, goat, horse, primate such as human and simian, bovine, and guinea pig, among others. The inventors have surprisingly found that these antibody fragments preserve the binding and specificity characteristics of the whole antibody while eliciting a lesser antibody reaction when administered to a subject of a different species. However, the simple preservation of the binding region of an antibody does not ensure that the binding characteristics of the antibody will be maintained. Antibodies are glycopolypeptides that are folded in certain specific conformations. When the glycosidic portion of the molecule is perturbed or excised, or portions of its amino acid sequence are eliminated, the folding pattern of the molecule is generally perturbed. Thus, any deletion of sequences of an antibody must be made taking into consideration that its folding-dependent properties may be diminished or even obliterated if the folding is affected, even though the amino acid sequences involved in the binding of the antigen are preserved.

The present inventors have selected the following strategy for the preparation and manufacture of the polypeptides and hybrid polypeptides of this invention. The cDNAs that encode the variable chains of an antibody may be obtained by isolation of mRNA from a hybridoma cell and reverse transcription of the mRNA, amplification of the cDNA by PCR and insertion of the DNA into a vector for sequencing and restriction enzyme cutting. The variable region cDNAs may then be PCR amplified, cloned, optionally into a vector carrying DNA sequences encoding a constant region(s), and sequenced, and then transfected

into a host cell for expression of the gene products. The binding specificity characteristics of the polypeptides may be then determined and compared to those of the whole antibodies.

Various polypeptide structures, such as Fab, Fab', $(Fab')_2$, variable fragments and combinations thereof having a desired specificity, may be constructed and may be bridged via a linker, or one or more of the polypeptides may be attached to one or more effector agent(s) or bridged via a linker. Multiple antibody variable regions, Fab, Fab', $(Fab')_2$, and the like, and combinations thereof, may also be constructed and bridged via linkers or attached to one or more effector agents such as are described below.

The cDNAs encoding the variable regions of the antibody of a desired specificity may also be cloned, e.g. into a vector that also contains sequences encoding an effector polypeptide such as whole antibodies, Fab, Fab', $(Fab')_2$ and human constant regions, enzymes, neuropeptides, other peptide transmitters, toxins, hormones, operative conjugation regions, cytokines, lymphokines and the like, under the same promoter. Although this is the cloning strategy utilized in the exemplary disclosure of this invention, other methods known in the art may also be utilized such as co-expression and the like.

In the exemplary disclosure provided herein, a human murine BrE-3 hybrid polypeptide binding to human mammary mucin and a human murine anti-KC-4 hybrid polypeptide binding to the KC-4 carcinoma antigen were constructed by joining the DNAs of the respective murine variable domains to the human constant polypeptide domains (an effector agent), then cloned into a hybrid vector, and the product expressed by transfecting the vector into myeloma cells.

Two polypeptides of the invention have been prepared comprising the variable regions of mouse antibodies BrE-3 (U.S. Patent No. 5,075,219) and KC-4 (U.S. Patent No. 4,708,930), and the kappa and $gamma_1$ constant region of a human antibody, and characterized by their molecular weights and binding specificities, and their binding constants determined and shown to be substantially the same as those of the respective parent mouse antibodies. Both polypeptides were shown to bind weakly to normal breast, lung, colon and endometrium, and strongly to carcinoma tissue sections by the ABC immunoperoxidase method. Their affinities for the human carcinoma antigen were determined. The portions of the variable polypeptides (mouse F_v regions) and effector agents (human F_c regions) were shown to be substantially identical to those of the non-human and human antibodies from which they were obtained.

The hybrid polypeptides of this invention possess less foreign antigenic epitopes that the foreign whole antibodies from which they are derived.

Accordingly, the inventors expect them to elicit a less complex immunogenic response in the species of the constant region, e.g. humans for the exemplary human-murine polypeptides, than the corresponding non-human whole antibodies. However, to what extent a portion of the heterologous, e.g. non-human, antibody could be deleted without altering the binding characteristics of the variable regions could not be predicted prior to this invention because of the substantial conformational alterations that normally occur upon truncation of amino acid sequences.

The present invention, thus, provides a pure, isolated polypeptide which selectively binds to an antigen on the surface or in the cytoplasm of neoplastic cells such as carcinoma cells or that is released by the cells, the polypeptide being selected from the group consisting essentially of at least one variable region of the light or heavy chains of an antibody having affinity and specificity for the HMFG antigen and an antigen found on the surface or the cytoplasm of the neoplastic cell or that is released by the cell; combinations thereof, wherein each polypeptide is operatively linked to at least one other polypeptide; and mixtures thereof.

The polypeptide of the invention may be as short as the shortest variable region of an antibody and as long as the variable region of an antibody plus other peptide sequences such as one or more antibodies, and the like, including non-peptide polymers of up to about 10^6 molecular weight, and in some instances even larger. The amino acid sequences of the variable regions of antibodies BrE-3 and anti-KC-4 are being described and were found to, by themselves or as a pair of light and heavy chains, bind to the respective antigens. When the polypeptide of the invention comprises combinations of variable regions that are operatively linked to at least one other region, the consecutive number of amino acids may exceed the number provided above. The smaller molecular weight polypeptides are particularly suitable for greater penetration of live cells, the brain-blood barrier, and tumors, among others, whereas the higher molecular weight polypeptides are better suited for in vitro or in vivo imaging and diagnosis.

The polypeptide of the invention may contain amino acid sequences derived from light and/or heavy chains of antibodies raised against a variety of antigens and/or epitopes. For example, the mouse antibodies disclosed in the examples were raised against human mammary fat globule (HMFG) mucin and the "KC-4" antigen of human carcinoma cells. Other antigens comprising a variety of epitopes may also be utilized as long as the antibody contributing the variable region displays affinity and specificity for the tumor cells such as carcinoma cells that will permit their selective binding to the cells in a variety

of tissues.

The heterologous antibody from which the polypeptide of the invention is derived may be a mouse, rat, goat, rabbit, human, guinea pig, horse, bovine, and primate including human and simian antibody, among others. The preparation of the antibody fragments encompassed by the polypeptides of the invention is similar whether the origin of the antibody is human or non-human. The original mRNA is obtained from cells of the desired species but the remainder of the work-up is similar.

In one particularly preferred embodiment of the invention, the anti-tumor polypeptide comprises the amino acid sequence ID No. 12 starting at amino acid D1, the amino acid sequence ID No. 13 starting at amino acid E1, the amino acid sequence ID Nos. 29 to 35, and the amino acid sequence ID Nos. 37 to 43, or combinations thereof wherein each polypeptide is operatively linked to at least one other polypeptide, or mixtures thereof. These sequences are shown in Tables 5, 13, and 14 below. The present polypeptides are provided either as a naked polypeptide or in glycosylated form. When provided in glycosylated form, the polypeptide may be operatively linked to a glycosyl residue provided by the eukaryiotic cell where it is expressed, or it may be cloned and expressed in a bacterial or other type of cell as the naked polypeptide, and the glycosyl residues added thereafter, for example by means of glycosyl transferases known in the art. Examples of glycosyl residues that may be added to the polypeptide of the invention are N-glycosylated and O-glycosylated residues, among others. The glycosyl residues added to the naked polypeptide may have a molecular weight of about 20 to 50,000 daltons, and more preferably about 20 to 20,000 daltons, depending on the size and molecular weight of the polypeptide to which they are attached. However, other types of polysaccharides and molecular weights may also be attached. The glycosyl residues may also be attached to the naked polypeptide of the invention by chemical means as is known in the art.

The heavy and light chain variable regions may be obtained individually or as V_H/V_L pairs, or attached to an effector peptide such as a constant region(s) or portions thereof, a drug, an enzyme, a toxin, a whole antibody, or any other molecule or radioisotope. There are advantages to using these molecular variants depending on the specific applications for which they are intended, some of which are listed below.

a) Smaller molecules penetrate target tissues more efficiently and are cleared from the body much more rapidly than larger molecules.

b) Single chain molecules can be manipulated and synthesized more efficiently that multiple chain molecules.

c) Many of these variants can be synthesized efficiently and inexpensively in bacteria.

d) Bi-functional or multifunctional molecules may carry polypeptide effectors, such as enzymes, toxins, radioisotopes, drugs, and other molecules, to a target tissue.

The following list encompasses exemplary polypeptides of the invention engineered with molecules derived from antibodies or antibody fragments. These polypeptides, among others, are suitable for the practice of this invention. A more extensive list of polypeptide constructs may be found in O'Kennedy, R., and Roben, P. (O'Kennedy, R., and Roben, P., "Antibody Engineering: an Overview", Essays Biochem. (England) 26:59-75 (1991)).

The polypeptides of this invention encompass variable regions with monoclonal antibodies, antibody fragments such as Fab, Fab', $(Fab')_2$, constant regions, single or multiple-domain and catalytic fragments, bi-functional or multifunctional combinations thereof, hybrid polypeptides thereof, enzymes, peptide hormones, molecules such as drugs and linkers, transmitters, and toxins, among others. These are suitable for imaging, therapy, diagnostics, and biosensors.

Single-Chain Antigen-Binding Polypeptides

Single chain antigen-binding polypeptides and their syntheses have been described, e.g., by Bird, R.E., et al. (Bird, R.E., et al., Science 242(4877):243-6 (1988); Bird, R.E., et al., Science 244(4903):409 (1989)).

For example, polypeptides such as V_H-linker-V_L and V_L-linker-V_H, have significant advantages over monoclonal antibodies in a number of applications. These may be expressed and purified from E. coli. The polypeptide linker binding the two chains may be of variable lengths. For example, about 2 to 50 amino acid residues, and more preferably about 12 to 25 residues, and may be expressed in E. coli.

Single Chain F_v ,(scF_v or sF_v)

Single chain antibodies consist of V_L and V_H with a polypeptide linker connecting the two chains (V_L-linker-V_H). The engineering may be done at the DNA level. So, knowledge of the sequence is required. These polypeptides have the conformational stability, folding, and ligand-binding affinity of single-chain variable region immunoglobulin fragments and may be expressed in Escherichia coli. (Pantoliano, M.V., et al., Biochem. (US) 30 (42):10117-25 (1991)). The polypeptide linker binding the two chains may be of variable length, for example, about 2 to 50 amino acid residues, and more preferably about 12 to 25 residues,

and may be expressed in E. coli. (Pantoliano, M.V., et al. (1991), supra).

A polypeptide such as an scF_v may be expressed and prepared from E. coli and used for tumor targeting. The clearance profiles for scF_v in some situations fragments are advantageous relative to those of normal antibodies, Fab or $(Fab')_2$ fragments. (Colcher, D., et al., J. Natl. Cancer Inst. 82 (14):1191-7 (1990)).

Another type of polypeptide comprises a V_H-linker-V_L and may have about 230 to 260 amino acids. A synthetic gene using E. coli codons may be used for expression in E. coli. A leader peptide of about 20 amino acids, such that of Trp LE may be used to direct protein secretion into the periplasm or medium. If this leader peptide is not naturally cleaved, the sF_v polypeptide may be obtained by acid cleavage of the unique asp-pro peptide bond placed between the leader peptide and the sF_v-encoding region. (Huston, J.S., et al., "Protein Engineering of Antibody Binding Sites: Recovery of Specific Activity in an Anti-Digoxin Single-Chain F_v Analogue Produced in E. coli.", P.N.A.S. (USA) 85 (16):5879-83 (1988)). The construction, binding properties, metabolism. and tumor targeting of single-chain F_v peptides derived from monoclonal antibodies may be conducted as previously described. (Milenic, D.E., et al., Cancer Res. (US) 51 (23 pt1):6363-71 (1991); Yokota, et al., "Rapid Tumor Penetration of a single-chain F_v and Comparison with Other Immunoglobulin Forms", Cancer Res. (US) 52(12):3402-8 (1992)).

This type of polypeptide provides extremely rapid tumor penetration and even distribution throughout tumor mass compared to IgG or Ig fragments Fab and $F(ab')_2$.

Bifunctional scF_v-Fxn or Fxn-scF_v

An example of this type of polypeptide is a V_L-linker-V_H effector peptide such as a hormone, enzyme, transmitter, and the like. These hybrid polypeptides may be prepared as described by McCarney, et. al. (McCarney, J.E. et al., "Biosynthetic Antibody Binding Sites: Development of a Single-Chain F_v Model Based on Antidinitrophenol IgA Myeloma MOPC 315", J. Protein Chem. (US) 10 (6):669-83 (1991)).

A bi-functional hybrid polypeptide containing an F_c-binding fragment B of staph protein A amino terminal to a single-chain F_v region of the present specificity is also encompassed and may be prepared as previously described. (Tai, M.s., et al., Biochem. 29 (35):8024-30 (1990)).

In this example of a hybrid polypeptide of this invention is a Staph. A fragment B (anti F_c)) - scF_v polypeptide. The order is backward of normal cases.

This FB-sF$_v$ may be encoded in a single synthetic gene and expressed as a polypeptide in E. coli. This polypeptide is a good example of a useful multifunctional targetable single-chain polypeptide.

A hybrid polypeptide also comprising antibodies to a human carcinoma receptor and antiogenin is also part of this invention. Antiogenin is a human homologue of pancreatic RNAse. This is an (Fab')$_2$-like antibody-enzyme peptide effector. Another hybrid polypeptide comprising a V_H-CH1 heavy chain-RNAse may be expressed in a cell that secretes a chimeric light chain of the same antibody. A secreted antibody of the similar structure was shown to cause the inhibition of growth and of protein synthesis of K562 cells that express the human transferrin receptor.(Rybak, S.M., et al., "Humanization of Immunotoxins", P.N.A.S. 89:3165-3169 (1992)).

Bi-specific Specific

A monoclonal anti-F_c antibody may be incorporated into a bi-specific F(ab')$_2$ derivative as described by Greenman, J., et al. (Greenman, J., et al., Mol. Immunol. (England) 28 (11):1243-54 (1991). A bi-specific F(ab')$_2$ comprising two (Fab'-(thioether-link)-Fab') chains provides the advantage that it is not blocked by human F_c gamma RII antibody. Thus, these are also utilized as effector agents herein. Bi-specific antibodies may be obtained when two whole antibodies are attached. Another way to obtain bi-specific antibodies is by mixing chains from different antibodies or fragments thereof. In this manner the "left" branch of the bi-specific antibody has one function while the "right" branch has another.

The new polypeptides in accordance with this invention may be screened with a filamentous phage system. This system may also be used for expressing any genes of antibodies or fragments thereof as well as for screening for mutagenized antibody variants as described by Marks, J.D., et al. (Marks, J.D., et al., "Molecular Evolution of Proteins on Filamentous Phage. Mimicking the Strategy of the Immune System", J.Mol. Biol. (England) 267 (23):160007-10 (1992)).

A library of V_H and V_κ genes may be cloned and displayed on the surface of a phage. Antibody fragments binding specifically to several antigens may be isolated as reported by Marks. J.D., (Marks, J.D., "By-Passing Immunization. Human Antibodies from V-gene Libraries Displayed on Phage", J. Mol. Biol. (England) 222 (3):581-97 (1991)).

Covalent Oligosaccharide Modifications

The present polypeptides alone or as hybrid polypeptides comprising

antibodies and fragments thereof may be, e.g., covalently modified utilizing oxidized oligosaccharide moieties. The hybrid polypeptides may be modified at the oligosaccharide residue with either a peptide labeled with a radioisotope such as ^{125}I or with a chelate such as a diethylenetriaminepentaacetic acid chelate with ^{111}In. The use of oligosaccharides provides a more efficient localization to a target than that obtained with antibodies radiolabeled either at the amino acid chain lysines or tyrosines.(Rodwell, J.D. et al., "Site-Specific Covalent Modification of Monoclonal Antibodies: In Vitro and In Vivo Evaluations", P.N.A.S. (USA) 83:2632-6 (1986)).

Several kinds of effector agents other than polypeptides may also be attached to the polypeptides of the invention. These include non-peptide polymers, monomers, atoms, etc. These are discussed below.

Particularly preferred polypeptides of this invention are those having the sequences ID Nos. 29 through 35, or 37 through 43, or combination thereof. Another preferred embodiment is that comprising amino acid sequence ID No. 12 starting at amino acid D1 or amino acid sequence ID No. 13 starting at amino acid E1, or combinations thereof. These amino acid sequences may be bound by a linker such as is known in the art. Examples of linkers are polylysine, EGKSSGSGSEJKVD, and (GGGGS)x3, and non-peptide polymers, among others.

Another preferred embodiment comprises a bi-functional polypeptide having a pair of light and heavy chains of the same specificity attached to one another by a linker, such as those provided above.

In another preferred embodiment, a bi-functional polypeptide comprises one set of light and heavy chains comprising at least one variable region, (e.g., amino acid sequences ID Nos. 12 or 13) and one set of light and heavy chain comprising at least one variable region, e.g., amino acid sequences ID Nos. 29 to 35 or 27 to 43, or combinations thereof. Multi-functional hybrid polypeptides may comprise several identical units or combinations of the above bi-functional polypeptides.

In another aspect, this invention provides an anti-tumor hybrid polypeptide that comprises at least one anti-tumor polypeptide and at least one effector agent operatively linked to the polypeptide, combinations thereof and mixtures thereof.

The effector agent utilized in this invention may be a peptide polymer other than the constant region of an antibody of the same species as the variable region, monomers, and atoms such as metals. In one particularly preferred embodiment, the effector agent may comprise an atom such a radioisotope, an enzyme or a fluorescent label. These effector peptides are suited for in vitro assays because they permit the identification of complexes formed by the

polypeptide of the invention. Radioisotopes are particularly preferred for in vivo imaging. Labeling of a polypeptide is known in the art (Greenwood, F.C., et al., Biochem. J. 89:114-123 (1963)). When a glycosylated polypeptide is utilized, the radiolabel may be attached to the glycosyl residue as is known in the art (Hay, G.W. et al, in Methods in Carbohydrate Chemistry, Vol 5:357, Whistler, R.L. Ed., Academic Press, NY and London (1965)). When the effector agent comprises a monomer, it may be a therapeutic, immunogenic or diagnostic agent, radioisotopes, DNA, or RNA monomers, chemical linkers, transmitter molecules, combinations thereof, or combinations thereof with peptide and non-peptide polymers or copolymers and atoms. Examples of therapeutic agents are antineoplastic drugs such as vincristine, intercalation drugs, adriamycin, enzymes, toxins and hormones, among others. Examples of immunogenic agents are other vaccines for carcinomas or for others purposes. Examples of diagnostic agents are radioisotopes and enzymes, among others.

Examples of therapeutic, immunogenic and diagnostic agents are toxins, vaccines, and radioisotopes, among others. Examples of radioisotopes are ^{111}In, ^{35}S, ^{90}Y, ^{186}Re, ^{225}Ac, ^{125}I and ^{99}Tc, among others. Examples of DNA and RNA monomers are A, T, U, G, C, among others. Examples of chemical linkers are dithiobis-(succinimidyl)propionate and bis-(sulfosuccinimidyl)suberate, among others. Examples of transmitter molecules are cAMP and cGMP, among others. Examples of toxins are ricin A-chain and abrin A-chain, among others.

When the effector agent is a non-peptide polymer linked to the polypeptide of the invention it may comprises an ester, ether, vinyl, amido, imido, alkylene, arylalkylene, cyanate, urethane, or isoprene polymers, DNA polymers, RNA polymers, copolymers thereof and copolymers thereof with peptide polymers or monomers, or have labeled atoms attached thereto. Examples of these are polyesters, polyethers, polyethyleneglycols, polyvinyls, polyamido and polyamido resins, polybethylenes, polytetrafluoroethylene, poly(ethylene)terephathalate, polypropylene, silicone rubber, isoprenes and copolymers thereof, copolymers of silicone and carbonated polylactic or polyglycolic acid or collagen, and the like. Particularly preferred are biodegradable and bioresorbable or bioabsorbable materials, which if detached from the polypeptide and left in the systemic circulation will not damage endogenous tissues.

The effector agent being a peptide may comprise antibodies such as IgG, IgM, IgE or IgD, the constant region of antibodies of a species different from the variable region or fragments thereof, and the variable regions, Fab, Fab', $(Fab')_2$ fragments of antibodies of the classes described above, hormones, enzymes, peptide transmitters and whole antibodies, combinations thereof, and

combinations thereof with non-peptide polymers, copolymers, monomers and atoms such as radioisotopes.

Examples of other antibodies, Fab, Fab', $(Fab')_2$, and variable regions thereof are those that specifically bind tumor epitopes, such as do BrE-3 and anti-KC-4, and others having specificities for different carcinoma epitopes, such as BrE-1. Br-E2, and Mc5, among others, and fragments thereof. Examples of peptide transmitters and hormones suitable for use herein are insulin, growth hormone, FSH, LH, endorphins, and TNF, among others. Examples of enzymes are peroxidase, LDH, alkaline phosphatase and galactosidase, among others.

In a particularly preferred embodiment, the polypeptide of the invention comprises non-human amino acid sequences, and the effector peptide comprises the constant region of the light or heavy chains of a human antibody or fragments thereof capable of binding anti-immunoglobulin, protein G or protein A, or fragments lacking this binding capability. This hybrid polypeptide is provided as a chimeric antibody if the polypeptide of the invention comprises the variable region of one species and the effector peptide comprises the constant region of another species. In one most preferred embodiment, the polypeptide comprises the murine-human chimeric antibodies expressed by the hybridoma cell lines having the ATCC Accession Nos. 11200 (Chimeric BrE-3 A1C10) and 11201 (Chimeric KC-4 1E8). These cells were deposited under the Budapest Treaty on November 13, 1992.

In one preferred embodiment, the hybrid polypeptide comprises two heavy and two light chains, each light and heavy chain comprising at least one variable region polypeptide of one species and the constant region of an antibody of a different species such as human, at least one other variable region, chimeric Fab , Fab' or $(Fab')_2$, fragments thereof, combinations thereof, and mixtures thereof. Still more preferred is a hybrid polypeptide comprising at least two murine-human chimeric antibodies or Fab , Fab' or $(Fab')_2$ fragments thereof operatively linked to one another. The peptide fragments may be covalently attached to one another as is known in the art (Marchis-Mouren G., et al., "HT 29, a Model Cell Line: Stimulation by the Vasoactive Intestinal Peptide (VIP); VIP Receptor Structure and Metabolism", Bioch. 70 (5):663-71 (1988)), or they may be synthesized by methods known in the art (Allen, G., et al., "Production of Epidermal Growth Factor in Escherichia Coli from a Synthetic Gene", J. Cell Sci. Suppl. 3:29-38 (1985)). In another preferred embodiment, the hybrid polypeptide of the invention described above having two heavy and two light chains operatively linked to one another is one where each pair of heavy and light chains has specificity for a different epitope. One example of this polypeptide is a pair of variableregion heavy and light chains of the BrE-3

polypeptide and a pair of variable region light and heavy chains of the KC-4 polypeptide of this invention that are covalently attached to one another by a peptide linker or by a non-peptide polymer. Non-peptide polymers may be covalently attached to peptides by methods known in the art (Duronio, V., et al., "Two Polypeptides Identified by Interleukin 3 Cross-Linking Represent Distinct Components of the Interleukin 3 Receptor", Exp. Hematol. 20 (4):505-11 (1992)).

In another embodiment, the invention provides a hybrid polypeptide comprising at least one variable region of the heavy chain of a first antibody operatively linked to a first effector agent, and at least one variable region of the light chain of a second antibody operatively linked to a second effector agent, wherein each pair of light and heavy chains has a predetermined specificity, combinations thereof, and mixtures thereof. In another preferred embodiment of the hybrid polypeptide, the at least one variable region of the heavy chain of the non-human antibody and the at least one variable region of the light chain of the non-human antibody are linked to one another by a non-peptide polymer or monomer. In still another preferred embodiment, the hybrid polypeptide of the invention is one wherein at least one pair of light and heavy chains comprising at least one variable region is linked to at least one other pair of light and heavy chains comprising at least one variable region. In a most preferred embodiment, the polypeptides and hybrid polypeptides of the invention have affinity and specificity for an epitope located in the most hydrophilic region of a 20 amino acid tandem repeat that makes up a large part of the polypeptide core of mammary mucin. A hexamer sequence APDTRP was shown to afford the strongest binding with five different monoclonal antibodies raised against human mammary fat globule (Mc1, Mc 5, BrE-1, BrE-2 and BrE-3). These monoclonal antibodies bind to overlapping polypeptide epitopes but have different tissue and tumor specificities, quantitatively differ in their binding to breast carcinoma cell lines when observed by flow cytometry and have different competition patterns for binding to the native antigen on breast carcinoma cells. Thus, preferred amongst the antibodies utilized for the preparation of the present polypeptide and hybrid polypeptide are those that exhibit strong binding to the hexamer peptide described above or to tandem repeats thereof.

This invention also provides an anti-tumor composition that comprises the anti-tumor polypeptide and/or hybrid polypeptide of the invention and a non-proteolytic carrier,e.g. a pharmaceutically-acceptable carrier. The anti-tumor polypeptide and hybrid polypeptide provided herein may be present in the composition in an amount of about 0.001 to 99.99 wt%, more preferably about 0.01 to 20 wt%, and still more preferably about 1 to 5 wt%. However, other

amounts are also suitable. Pharmaceutically-acceptable carriers are known in the art and need not be further described herein and may be provided in a separate sterile container or in admixture with the polypeptide. Typically, saline, aqueous alcoholic solutions, albumin-saline solutions, and propylene glycol solutions are suitable. However, others may also be utilized. When utilized for therapeutic purposes, the composition may also contain other ingredients as is known in the art. Examples of these are other anti-neoplastic drugs such as adriamycin, and mitomycin, among others, cytoxan, PALA and/or metrotrexate. However, other therapeutic drugs, diluents, immunological adjuvants and the like may be also be added. When the composition described above is utilized for in vivo imaging, it may comprise about 0.001 to 99.9 wt% polypeptide, and more preferably about 0.01 to 20 wt% polypeptide. Typically, when the composition is utilized for therapeutic purposes it may contain about 0.001 to 99.9 wt% polypeptide, and more preferably about 0.01 to 20 wt% polypeptide. When utilized for the in vitro diagnosis of carcinomas the composition of the invention may comprise about 0.001 to 30 wt% polypeptide, and more preferably about 0.001 to 10 wt% polypeptide. Other amounts, however, are also suitable. Such products find one utility in the treatment of tumors such as of the breast, lung, ovary, endometrial, pancreas, prostate and colon tumors, among others. The present polypeptides are particularly suitable for repeated administrations to a patient and for long term therapies such as is the case of metastases and/or reoccurrence of tumors.

Also provided herein is a tumor diagnostic kit, that comprises the composition of the invention comprising the anti-tumor polypeptide, a solid support, anti-tumor antibody, anti-constant region immunoglobulin, protein G or protein A, and instructions for its use. This diagnostic kit may be utilized by covalently attaching the polypeptide of the invention to the solid support by means of a linker as is known in the art. In a particularly preferred embodiment, the support is coated with methylated albumin as described in US Patent No. 4,572,901, the relevant text of which being incorporated herein by reference. When a biological sample is added to a well, the polypeptide of the invention will bind to tumor antigens present in the biological sample. If a competitive assay is utilized, to the solid supported antigen are added a known amount of the polypeptide and the sample. Thereafter, labeled anti-constant region immunoglobulin, protein G or protein A in labeled form may be added for detection.

The anti-tumor antibody may be obtained by challenging a human or non-human animal with tumor cells such as human carcinoma cells, the human milk fat globule mucin and the like, as is know in the art (Peterson, J.A., et al.,

Hybridoma 9:221 (1990)). Other antibodies may be prepared similarly by varying the antigen and/or the species utilizied. If the antibody utilized is monoclonal, it may be prepared as described by Kohler, G. and Milstein, C. (Kohler, G. and Milstein, C., "Continuous Culture of Fused Cell Secreting Antibody of Predefined Specificity", Nature 256:495-497 (1975)). Suitable for use in this invention are antibodies such as IgG, IgM, IgE and IgD. Protein A , protein G and anti-constant region immunoglobulin may be obtained commercially.

Another diagnostic kit for tumors such as carcinomas comprises the anti-tumor composition described above, wherein the effector agent of the hybrid polypeptide comprises an enzyme, a radioisotope, a fluorescent label and/or a peptide comprising the constant region of an antibody of a different species or fragments thereof capable of binding immunoglobulin, protein G or A, anti-tumor antibody, immunoglobulin, protein G or protein A, a solid support having operatively linked thereto an antigen which specifically binds to the anti-tumor hybrid polypeptide of the invention and the antibody, and instructions for its use.

When the effector agent comprises a peptide, such as the constant region of an antibody of a different species, the solid support may have operatively linked thereto an antigen which specifically binds to the effector peptide. This permits the binding of the anti-tumor polypeptide to the antigen molecule attached to the solid support. Any complex formed between the hybrid polypeptide of the invention and the supported antigen will, thus, remain attached to the solid substrate. A competitive assay may then be conducted by addition to the solid supported antigen of a known amount of the antigen hybrid polypeptide and the sample. The amount of neoplastic antigen present in the sample may be obtained from a dilution curve by addition of immunoglobulin, protein G or protein A, e.g., labeled, to bind the hybrid polypeptide that is now attached to the support. This kit may be used in a competitive assay where the supported antigen molecule competes with neoplastic antigen in the sample for a known amount of the polypeptide of the invention. The assay was described by Ceriani, R.L., et al. (Ceriani, R.L., et al., Anal. Biochem. 201:178-184 (1992)), the relevant text thereof being incorporated herein by reference.

Still part of this invention is an in vivo method of imaging and/or diagnosing a tumor that comprises administering to a subject suspected of carrying a neoplastic tumor the anti-tumor polypeptide of the invention in radiolabeled form, in an amount effective to reach the tumor and bind thereto, and detecting any localized binding of the labeled polypeptide to the tumor. Typically, the polypeptide of the invention may be administered in an amount of about 0.01 to 100 μg/kg weight per treatment, and more preferably about 0.1

to 50 μg/kg weight per treatment. However, other amounts may also be utilized. Radiolabels that may be utilized are ^{111}In, ^{35}I, ^{99m}Tc, and ^{131}I, among others. These radioisotopes may be detected with a PET scanner, NMR imaging, and radioactivity counting apparatus that are in wide use by the medical community.

Also provided herein is an in vitro method of diagnosing a neoplasia that comprises contacting a biological sample with the anti-tumor polypeptide or hybrid polypeptide of the invention to form an anti-tumor polypeptide-antigen complex with any neoplastic cell antigen present in the sample, and detecting any complex formed. The biological sample is typically obtained from a subject suspected of being afflicted with the tumor. Suitable biological samples are serum, blood, sputum, feces, lymph fluid, spinal fluid, lung secretions, and urine, among others. Clearly, any source of fluid, tissue and the like may be prepared for use in this method as is known in the art.

The hybrid BrE-3 polypeptide of the invention and the murine BrE-3 antibody show substantially no strong binding to normal tissue. The hybrid BrE-3 polypeptide shows a binding pattern similar to that of the murine BrE-3 antibody. The murine BrE-3 antibody was shown to bind strongly with specificity to carcinoma tumors of the breast, lung, ovary, bladder, and the endometrium, mesothelioma, colon, kidney, liver, merkel cells, pancreas, salivary glands, sarcomas and thyroid, among others. Only weak binding was shown to normal breast tissue, lung tissue, distal convoluted tubes of the kidney, acini of the pancreas and stomach mucosa (Peterson, J.A., et al. (1990), supra). The anti-KC-4 hybrid polypeptide has tissue specificity similar to that of the murine anti-KC-4 antibody. The anti-KC-4 monoclonal antibody was shown to bind specifically and strongly to solid tumor tissue in the lung, colon, kidney, breast, stomach, prostate, pancreatic, lymph node doctal and lymphoma, and non-specifically and weakly to normal breast, kidney, and stomach tissue. KC-4 also showed some weak binding to normal tissue including spinal cord, uterus, thyroid, tongue, prostate, spin, adrenal, lung, gall bladder, heart, lymph nodes, colon, liver, brain, testes, thymus, and placenta (U.S. Patent No. 4,708,930). In one preferred embodiment of the in vitro diagnostic method, the anti-tumor polypeptide added to the biological sample comprises a labeled hybrid polypeptide. Suitable labeling materials were described above. This method may be practiced with the solid support containing kit described above, as a competitive assay as disclosed by Ceriani, R.L., et al.(Ceriani, R.L., et al. (1992), supra).

Also provided herein is a method of inhibiting the growth or reducing the size of a primary or metastasized human carcinoma comprising administering to

a human in a need of the treatment an effective amount of the anti-tumor hybrid polypeptide of the invention. Typically, the hybrid polypeptide may be administered in an amount of about 0.001 to 200 μg/kg body weight per treatment, and more preferably about 0.01 to 100 mg/kg body weight per treatment. However, other amounts are also suitable. Generally, the administration of the hybrid polypeptide is conducted by infusion so that the amount of radiolabel, toxin or other effector agent present that may produce a detrimental effect may be kept under control by varying the rate of administration. Typically, the infusion of one dose may last a few hours. However, also contemplated herein is the constant infusion of a dose for therapeutic purposes that will permit the maintenance of a constant chronic level of the hybrid polypeptide in serum. The infusion of the hybrid polypeptide of the invention may be conducted as follows. Intravenous (I.V.) tubing may be pretreated, e.g., with 0.9 % NaCl and 5% human serum albumin and placed for intravenous administration. The prescribed dose of the polypeptide may be infused as follows. Unlabeled antibody may be infused initially. 30 minutes after completion of the unlabeled antibody infusion, ^{111}In-labeled and Y-90 labeled antibody may be co-infused. The I.V. infusion may comprise a total volume of 250 ml of 0.9 % NaCl and 5 % human serum albumin and be infused over a period of about 2 hours depending on any rate-dependent side effects observed. Vital signs should be taken every, e.g., 15 minutes during the infusion and every one hour post infusion until stable. A thorough cardiopulmonary physical examination may be done prior to, and at the conclusion, of the infusion. Medications including acetaminophen, diphenhydramine, epinephrine, and corticosteroids may be kept at hand for treatment of allergic reactions should they occur. The administration of the hybrid polypeptide of the invention may be repeated as seen desirable by a practitioner. Typically, once a first dose has been administered and imaging indicates that there could be a reduction in the size of the tumor, whether primary or metastasized, repeated treatments may be administered every about 1 to 100, and more preferably about 2 to 60 days. These repeated treatments may be continued for a period of up to about 2 years, and in some circumstances even for longer periods of time or until complete disappearance of the tumor(s). The administration of the hybrid polypeptides of this invention is typically more useful for therapeutic purposes when a primary tumor has, for example, been excised. Thus, it is primarily, for mopping up after surgical intervention or in cases of cancerous metastases that the present method is of most use.

Also provided herein is a pure, isolated polydeoxyribonucleotide that comprises a oligodeoxyribonucleotide encoding the polypeptide or hybrid

polypeptide of this invention, including all redundant sequences. In one preferred embodiment, the polydeoxyribonucleotide of the invention comprises a DNA sequence selected from the group consisting of DNA Sequence ID No: 10 starting at codon 20 (GAT), DNA Sequence ID No: 12 starting at codon 20 (GAA), DNA Sequence ID No: 26, codon 20. Also preferred is Sequence ID NO: 27, starting at codon 20. The above DNA sequences may be cloned for expression under the same operon.

Also provided herein in is a hybrid vector that comprises a vector having the polydeoxyribonucleotide of this invention operatively linked thereto. Typically, vectors capable of replication both in eukaryotic and prokaryotic cells are suitable. When the preparation of a glycosylated polypeptide is desired the vector should be suitable for transfection of eukaryotic host cells. In one preferred embodiment, the hybrid vector further comprises a polydeoxyribonucleotide comprising an oligodeoxyribonucleotide encoding an effector peptide, the effector peptide-encoding polydeoxyribonucleotide being operatively linked to the vector. As already indicated, the various DNA sequences may be cloned for expression under the same operon. In addition, the polydeoxyribonucleotide encoding the effector polypeptide may also be cloned for expression under the same operon. In one preferred embodiment, the invention provides a hybrid vector, wherein the oligodeoxyribonucleotide encodes a polypeptide selected from the group consisting of the variable regions of the heavy and the light chains of the antibody and combinations thereof, and the effector peptide-encoding oligodeoxyribonucleotide is operatively linked to the oligodeoxyribonucleotide so as to express a hybrid polypeptide comprising at least one variable region of the light or heavy chains of the polypeptide or combinations thereof, and the effector peptide. Other polypeptides and hybrid polypeptides comprising effector peptides described above may also be prepared as described above.

This invention also encompasses a host cell that has been transfected with the hybrid vector described above. Suitable hosts are prokaryotic and eukaryotic hosts such as bacteria, yeast, and mammalian cells such as insect cells and non producing hybridoma cells, among others. Suitable vectors and/or plasmids for the transfection of each one of these types of hosts are known in the art and need not be further described herein. Also known in the art are methods for cloning DNA sequences into each one of these types of vectors and for transfecting the different types of host cells. Particularly preferred are the cell lines having the ATCC Accesion Nos. 11200 (Chimeric Br-E 3 A1C10) and 11201 (Chimeric KC-4 1E8).

Polyribonucleotides may be obtained by transcription of the

polydeoxyribonucloetides described above as is known in the art. Provided herein are polyribonucleotides comprising oligoribonucleotides encoding at least one antibody variable region, combinations thereof, and combinations thereof with an effector peptide may be prepared by cloning the desired DNA segments and then transcribing the thus obtained polydeoxyribonucleotide into the corresponding RNA sequences.

This invention also provides a method of producing a polypeptide which selectively binds to the HMFG antigen and to an antigen present on the surface or in the cytoplasm of tumor cells such as carcinomas or that is released by the cells, that comprises cloning the polydeoxyribonucleotide of the invention into a vector to form a hybrid vector, transfecting a host cell with the hybrid vector and allowing the expression of the anti-tumor polypeptide, and isolating the anti-tumor polypeptide or mixtures thereof. Preferably, the cloning and transfecting steps are conducted by cloning polydeoxyribonucleotides encoding polypeptides selected from the group consisting of at least one of variable region of the heavy or light chains of the human antibody. The method may further comprise allowing the expressed polypeptides to interact with one another to form double chain polypeptides, each chain polypeptide comprising at least one variable region of the light or heavy chain of the antibody. Still part of this invention is a method of producing an anti-tumor hybrid polypeptide comprising an effector peptide and a polypeptide which specifically binds to an antigen on the surface or in the cytoplasm of human carcinoma cells or that is released by the cells, the method comprising transfecting a host cell with the hybrid vector of this invention carrying a DNA sequence encoding the hybrid polypeptide, allowing the expression of the anti-tumor hybrid polypeptide, and isolating the anti-tumor hybrid polypeptide or mixtures thereof. The techniques for obtaining mRNA, conducting reverse transcription and PCR amplification of DNA, cloning DNA sequences into a vector, transfecting a host cell, and purifying polypeptides from a culture medium are known in the art and need not be further described herein.

This invention also encompasses an anti-idiotype polypeptide that comprises polyclonal antibodies raised against the polypeptide of the invention, monoclonal antibodies thereof, fragments thereof such as Fab, Fab', $(Fab')_2$, and variable region fragments, an oligopeptide comprising the amino acid sequence APDTRPA or tandem repeats thereof, combinations thereof wherein each oligopeptide, antibody or fragment thereof, is operatively linked to at least one other oligopeptide, antibody or fragment thereof, and mixtures thereof. In one particularly embodiment, the anti-idiotype polypeptide of the invention specifically binds to at least one variable region of the light or heavy chains of the anti-tumor polypeptide of this invention. Techniques for obtaining anti-

idiotype polypeptides is known in the art and need not be further described herein (Nisonoff, A. and Lamoyi, "Implication of the Presence of an Internal Image of an Antigen in Anti-Idiotype Antibodies: Possible Applications to Vaccine Production", Clin. Immunol. Imminopathol. 21:397-406 (1981)). Moreover, the technique for expressing hybridomas producing monoclonal antibodies of a certain specificity is also known in the art (Kohler, G. and Milstein, C. (1975), supra). Techniques for obtaining different antibody fragments were described above or are known in the art and need not be further described herein (Wilbanks, T., et al., "Localization of Mammary Tumors In Vivo with ^{131}I-Labeled Fab Fragments of Antibodies Against Mouse Mammary Epithelial (MME) Antigens", Cancer 48:1768-1775 (1981)). In one particularly preferred embodiment, the anti-idiotype polypeptide of the invention comprises an effector agent operatively linked to the polypeptide. Effector agents suitable for use herein described above for the anti-tumor polypeptide of the invention are also suitable for use with the anti-idiotype polypeptide. Preferred are polyclonal antibodies raised against the polypeptide of the invention, and a monoclonal antibody obtained by fusion of a B-cell producing an antibody having specificity for the polypeptide of the invention and an immortalized cell line. Also preferred are fragments of the monoclonal antibody such as Fab, Fab', $(Fab')_2$ and variable region fragments. Also preferred are combinations of the above fragments and combination of the fragments with whole antibodies. In another preferred embodiment, the anti-idiotype polypeptide comprises the hexapeptide or tandem repeats thereof.

Also provided herein is an anti-tumor vaccine, that comprises the anti-idiotype polypeptide of the invention, and a pharmaceutically-acceptable carrier. Typically, the anti-idiotype polypeptide is present in the composition in an amount of about 0.001 to 99.99 wt%, and more preferably about 0.01 to 50 wt% of the composition. However, other amounts are also suitable. Pharmaceutically-acceptable carriers are known in the art and need not be further described herein. The vaccine provided herein may further comprise other ingredients such as adjuvants, and the like. Examples of adjuvants are SAF-1 and Freund's, among others. Suitably, other ingredients typically used for the preparation of vaccines may also be utilized herein. In one embodiment, the vaccine of the invention may be provided in unit form as a powder or in a diluent. In another embodiment, it may be provided in powder form in a sterile container comprising a plurality of doses for preparation prior to utilization. Diluents that are suitable for the preparation of a formulation that may be administered to a patient by injection are known in the art. Examples were provided above. A vaccination kit against tumors is also provided by this

invention that comprises, the vaccine described above and a diluent, in separate sterile containers, and instructions for its use. Also provided herein is a method of vaccinating against tumors that comprises administering to a subject an effective amount of the anti-idiotype polypeptide described above. Typical amounts administered to a human are about 0.001 to 500 μg/kg body weight/dose, and more preferably about 0.01 to 100 μg/kg body weight/dose. The anti-idiotype vaccine of the invention may be administered repeatedly in order to boost the active immunization produced by the first dose. An anti-idiotype antibody very likely resembles the epitope on the carcinoma cell to which the anti-tumor antibody binds. Thus, it may be utilized for the production of an immunological response by the patient against its own tumor cells.

When an anti-idiotype polypeptide of non-human origin is administered to a human, it may produce some detrimental response. Accordingly, in theory, the smaller the non-human amino acid sequence the anti-idiotype polypeptide contains, the lesser the immunogenic response it will elicit in a human. Accordingly, preferred anti-idiotype polypeptides for use in humans are those containing at least one variable region of a non-human antibody binding selectively to the anti-tumor polypeptide described herein. Also preferred are human anti-idiotype antibodies, variable fragments thereof, and fragments thereof that are operatively linked to an effector agent comprising a human polypeptide that may include the constant region of a human antibody and fragments thereof, non-peptide polymers, monomers and atoms that may be radiolabeled as described above. Other types of constructs are also possible, several of which were described above.

In addition to the utilization of the hexamer APDTRP peptide for producing antibodies that strongly bind to tumor cells, the APDTRP hexamer may also be utilized for clearing from the circulation of a patient antibody molecules such as the polypeptide of the invention which have been used for therapeutic purposes. The hexapeptide may be utilized as a tandem repeat comprising up to about 10,000 repeats of the basic unit, and in some instances up to about 500,000 repeats. In another embodiment, one or more hexapeptides may be operatively linked to other polypeptide sequences of related or unrelated function, which sequences provide bulk that aids the clearance through the liver and/or kidneys of the immunological complex formed between the circulating unbound or residual antibody or polypeptides utilized for the therapy of carcinomas and the hexapeptide. In the absence of such treatment, the therapeutic antibody, which may carry a radioisotope, a toxin or other therapeutic molecules, may remain in the circulation for several days and in some instances weeks. This, in the case of a radioactively labeled antibody or

polypeptide of the invention may produce extensive damages, particularly to the bone marrow, which are highly detrimental to the health of the patient, and in some instances lethal. Thus, this invention also provides a method of lowering the serum concentration of the circulating polypeptide that binds to an antigen found on the surface or in the cytoplasm of a neoplastic cell or that is released by the cell comprising administering to the subject a binding polypeptide selected from the group consisting of an oligopeptide comprising the amino sequences APDTRPA, tandem repeats thereof, and the anti-idiotype polypeptide described above, in an amount effective to bind the circulating polypeptide, to thereby accelerate its clearance. In one preferred embodiment a tandem repeat of up to about 20,000 and even up to about 50,000 APDTRPA hexapeptides is preferred. Another preferred embodiment comprises an oligopeptide comprising one or more of the hexapeptide sequences and having a molecular weight of about 5,000 to 1,000,000. Still another preferred embodiment is that where the polypeptide comprises the anti-idiotype polypeptide of this invention. Typically, the binding polypeptide is administered in an amount of about 0.01 to 100.00 μg/kg body weight/dose, and more preferably about 1 to 25 μg/kg body weight/dose. However, other amounts may also be utilized. The administration of the anti-idiotype polypeptide may be infusion intra-muscularly or subcutaneously.

Also provided herein is a method of inhibiting the growth or reducing the size of a primary or metastasized tumor comprising administering to a subject, e.g. a human, in need of the treatment an effective amount of an anti-tumor hybrid polypeptide comprising an effector agent selected from the group consisting of radioisotopes, therapeutic drugs and vaccines, and an anti-tumor polypeptide which selectively binds to the HMFG antigen and to an antigen on the surface or in the cytoplasm of neoplastic cells or that is released by the cells, allowing the hybrid polypeptide to reach the tumor and the polypeptide to bind thereto, and administering to the subject an amount of the anti-idiotype polypeptide of the invention effective to bind residual or unbound circulating hybrid polypeptide to thereby accelerate the clearance of the hybrid polypeptide.

The present polypeptide and hybrid polypeptide are thus useful for determining in vitro the presence of neoplastic cells in a fluid or in tissue samples by histochemistry, for imaging tumors in vivo, for ex vivo purging neoplastic cells from biological samples such as spinal fluid and the like, as well as for diagnostic applications.

Having now generally described this invention, the same will be better understood by reference to certain specific examples, which are included herein for purposes of illustration only and are not intended to be limiting of the

invention or any embodiment thereof, unless so specified.

EXAMPLES

Example 1: Methods Utilized

The procedures utilized herein for the reverse-transcription (RT) of RNAs encoding the variable regions and the subsequent amplification of the cDNAs by the polymerase chain reaction (PCR) have been described (Orlandi, R., et al., "Cloning Immunoglobulin Variable Domains for Expression by the Polymerase Chain Reaction", P.N.A.S. (USA) 86:3833-3837 (1989); Coloma, M.J., et al., "Primer Design for the Cloning of Immunoglobulin Heavy-Chain Leader-Fvs from Mouse Hybridoma Cells Using the PCR", Bio.Techniques 11:152-156 (1991); Gavilondo-Cowley, J.V., et al., "Specific Amplification of Rearranged Immunoglobulin Fv Genes from Mouse Hybridoma Cells", Hybridoma 9:407-417 (1990)).

Total RNA is an adequate substrate for RT-PCR. Polyadenylated RNA was utilized herein, however, because it contains only minor levels of contaminating ribosomal RNA and practically no DNA. The polyadenylated RNA was isolated with a Fast Track mRNA isolation kit (Invitrogen Corporation, San Diego, CA).

The oligonucleotides were synthesized on a PCR-Mate EP DNA synthesizer model 391 (Applied Biosystems, Foster City, CA). A PCR mouse Ig primer set was purchased from Novagen (Madison, WI), and complementary DNA (cDNA) was prepared with an RNA PCR kit (Perkin Elmer-Cetus, Norwalk, CT).

PCR DNA fragments were cloned directly into pCR1000, using a TA cloning kit (Invitrogen Corporation, San Diego, CA). Plasmid DNA was isolated with a kit purchased from Qiagen (Tchapsworth, CA), and DNA sequencing was conducted with a Sequenase 2.0 DNA sequencing kit (United States Biochemical, Cleveland, Ohio) using aqueous 5'α-^{35}SdATP at 600 mCi/mmol (Amersham Corporation, Arlington Heights, Il).

Sequence analyses were performed on a Macintosh computer using the program GeneWorks (IntelliGenetics, Inc, Mountain View, CA).

Example 2: Tissue Culture Media

SP2/0-Ag14 cells (Shulman, M., et al. (1978), below) were cultured either in Dulbecco's modified Eagle's medium (DME): fetal bovine serum (FBS), 90:10 (v/v) or in a mixture of DME:RPMI:FBS, 45:45:10 (v/v/v) or RPMI:FBS,

90:10 (v/v). Penicillin and streptomycin were added to prevent bacterial growth. When serum-free medium was utilized, it contained an HL-1 supplement as directed by the manufacturer (Ventrex Labs., Portland, ME). The freezing medium was 10% DMSO in bovine serum.

Example 3: PCR Primers

Primers and primer mixtures MulgκV$_L$5'-C, MulgλV$_L$3'-1, MulgV$_H$5'-C, MulgV$_H$5'-F, and MulgγV$_H$3'-2 were part of a primer set purchased from Novagen. Their sequences may be obtained from Novagen. Other primers were synthesized by the inventors. These sequences are shown in Table 1 below.

Table 1: Synthetic Primers

JO2- T GAA GCT TGC TCA CTG GAT GGT GGG AA (Seq. ID No: 1);
JO3- AGA TGG GGG TGT CGT TTT GG (Seq. ID No: 2);
JO4- GCT TGA ATT CCA GGG GCC AGT GGA TAG A (Seq. ID No: 3);
V$_H$1BACK (*) - AG GT(CG) (CA)A(GA) CTG CAG (CG)AG TC(TA) GG (Seq. ID No: 4);
JO14- ATG TAC TTG GGA CTG AAC TAT GTC TT (Seq. ID No: 5).

* Orlandi, R., et al. (Orlandi, R., et al. "Cloning Immunoglobulin Variable Domains for Expression by the Polymerase Chain Reaction", P.N.A.S. (USA) 86: 3833-3837(1989)).

Example 4: Cloning of Chimeric BrE-3 Antibody Polydeoxyribonucleotide

Two expression vectors pAG4622 and pAH4604 were utilized herein (Coloma, M.J., et al., "Novel Vectors for the Expression of Antibody Molecules Using Variable Regions Generated by PCR", J. Immunol. Methods 152:89-104 (1992)). These were kindly provided by S.L. Morrison (Dept. of Microbiology and Molecular Genetics, UCLA). The construction and expression of chimeric genes was performed as described by Coloma, M.J., et al. (Coloma, M.J., et al. (1992), supra).

Oligonucleotides were synthesized and used in a PCR mixture to produce variable heavy (V$_H$) and variable light (V$_L$) fragments with the correct ends for insertion into the pAG4622 and pAH4604 expression vectors. There sequences are shown in Table 2 below.

Table 2: Synthesized Oligonucleotides

JO16 (senseV$_H$ leader)
GGG GATATC CACC ATG TAC TTG GGA CTG AAC TAT GTC TTC A (Seq. ID No: 6)

JO17 (sense V$_L$ leader)
GGG GATATC CACC ATG AAG TTG CCT GTT AGG CTG TTG GT (Seq. ID No: 7)

JO18 (anti-sense JH3)

GGG GCTAGC TGC AGA GAC AGT GAC CAG AGT CC(Seq. ID No: 8)
JO19 (anti-sense Jκ1)
GGG GTCGACTTAC G TTT GAT TTC CAG CTT GGT GCC TCC A (Seq. ID No: 9)

The original pCR1000 clones were utilized as the starting templates for the PCR. The new PCR products were cloned back into pCR1000 and their sequence confirmed. Correctly modified and amplified fragments were excised with either EcoRV and Sal I (for V_L) or with EcoR V and Nhe I (for V_H). These fragments were then ligated into the respective vectors, which had been cut open with the appropriate restriction enzymes. Both the vectors and the inserts were purified from an agarose gel prior to ligation, using the Bio101 GeneClean kit (glass beads) (La Jolla, CA).

Example 5: Expression of Mouse-Human Chimeric Antibody

The V_H and V_L regions in the final mouse-human chimeric antibody were sequenced once again to verify that their sequences were correct.

The non-producer myeloma cell line SP2/0-Ag14, (ATCC: CRL 1581, Shulman, M., et al., "A Better Cell Line for Making Hybridomas Secreting Specific Antibodies", Nature 276:269-270, (1978)) was transfected, and a chimeric antibody were isolated as described by Coloma, M.J. et al. (1992), with the following modification. The selection was only undertaken for the uptake of hisD by adding 5mM histidinol to the medium and readjusting the pH to 7.4 with NaOH.

Example 6: Production of Transfected Hosts

After ten days, the stable transfectant colonies were clearly established at a frequency of approximately 10^{-5}. The colonies were transferred to a normal medium (without histidinol) and the supernatants from stable transfectants were assayed for the presence of the mouse-human chimeric BrE-3 antibody. This was done by capturing the secreted mouse-human chimeric BrE-3 antibody with a plate-bound goat anti-human-κ antibody and developing with goat anti-human-γ antibody as described by Coloma, M.J. et al. with the following modification. The secondary antibody utilized herein was radiolabeled with ^{125}I.

Example 7: Confirmation of Mouse-Human Chimeric BrE-3 Antibody Expression

The supernatants were assayed for binding to human milk fat globule (HMFG) as described by Ceriani R. L. (Ceriani R.L., et al., Diagnostic Ability of Different Human Milk Fat Globule Antigens in Breast Cancer", Breast Cancer Res. Treat.

15:161-174 (1990)). HMFG was bound to the microtiter plates as described previously (Ceriani R.L., "Solid Phase Identification and Molecular Weight Determination of Cell Membrane Antigens with Monoclonal Antibodies", in: Monoclonal antibodies and functional cell lines. Progress and application, Bechtol, K.B., McKern, T.J., and Kennett, R., Eds., Plenum Press, New York, pp 398-402 (1984)).

Most colony supernatants were positive by both assays. The colonies that secreted the highest level of chimeric antibody into the supernatants, as determined by these assays, were subcloned and subsequently adapted to serum-free medium for the purification of antibody.

Example 8: Competition Assay

The antibody-antigen affinity constants for the mouse-human chimeric antibody which binds to human milk mucin and the whole murine antibody were determined by obtaining the reciprocal value of the concentration of competing unlabeled monoclonal antibody giving 50% binding as described by Sheldon, K. et al. (Sheldon, K.,et al., "Characterization of Binding of Four Monoclonal Antibodies to the Human Ovarian Adenocarcinoma Cell Line HEY", Biochem. Cell Biol., 65: 423-428, (1987)). The protocol for the assay was as follows.

Microtiter plates (Dynatech, Chantilly, VA) were prepared using successive layers of methylated BSA, glutaraldehyde, anti-β-galactosidase and the bacterial fusion protein 11-2 (a hybrid of β-galactosidase and human mammary mucin) as described in Ceriani, R.L., et al. (Ceriani, R.L., et al., "A Novel Serum Assay for Breast Cancer Epithelial Antigen Using a Fusion Protein", Anal. Biochem. 201:178-184 (1992). Each well contained 388ng of the 11-2 fusion protein. To each well were added 25μl ^{125}I -BrE-3 (ATCC No. HB 10028) in RIA buffer (10% bovine calf serum, 0.3% triton X-100, 0.05% sodium azide pH7.4, in phosphate buffer saline), and competed with 25 μl of either unlabeled murine antibody or mouse-human chimeric antibody in RIA buffer at final concentrations in the nanomolar range.

Iodinations were performed with ^{125}I (17 Ci/mg, Nordion International Inc., Kanata, Ontario, Canada). 50 micrograms of monoclonal antibody BrE-3 (Coulter, Hialeah, FL) were labeled at a specific activity of 9.56 mCi/mg using the chloramine T method as described by Ceriani, R.L. and Blank, E.W., (Ceriani, R.L., and Blank, E.W., "Experimental Therapy of Human Breast Tumors with 131I-Labeled Monoclonal Antibodies Prepared Against the Human Milk Fat Globule", Cancer Res. 48:4664-4672 (1988)).

When the counts of bound radiolabeled murine BrE-3 antibody were plotted an the Y axis and the logarithm of the nanomolar (nM) concentration of

competing unlabeled murine BrE-3 antibody or mouse-human chimeric antibody were plotted in the X axis, both curves overlapped within 5% error (Figure not shown).

This proves that the variable region's affinity characteristics have been preserved.

Example 9: Amplification of cDNAs Encoding BrE-3 Variable Regions

The cDNAs that encode the BrE-3 mouse immunoglobulin variable domains (V_H and V_L) were prepared by reverse transcription and PCR amplification (RT-PCR) from polyadenylated RNA isolated from 10^8 BrE-3 hybridoma cells by the following procedure.

The JO2, JO3, JO4, JO14 and V_H1BACK primers were synthesized, and there sequences shown in Example 3 above. Other primers were purchased from Novagen. With the exception of V_H1BACK, which is a framework-specific primer, all sense primers are specific for the leader peptide region. All anti-sense primers are specific for the constant regions. The degenerate λ chain of the specific primer MulgλV_L3'-1 (from Novagen), was used to isolate the κ chain cDNA clones because of the similarity of the λ and κ. An identical κ chain clone was isolated with primer JO2 which is specific for the κ chain constant domain. The V_H region cDNA could not be isolated with the available leader peptide primers. Thus, the V_H1BACK primer was used, which yielded the V_HcDNA γ72. The leader-peptide primer JO14 was then designed by extrapolating from the framework sequence of γ72, using cataloged nucleotide sequences (Kabat, E.A., et al., "Sequences of Proteins of Immunological Interest", U.S. Dept. Health and Human Services, NIH publication No. 91-3242, 5th. Edition (1991). After sequential PCR reactions, this new primer yielded the complete V_H framework cDNA. This information is summarized in Table 3 below.

Table 3: Primer Combinations for PCR Amplification

	Clone No.	Sense Primers	Antisense Primers
V_L	152	MulgκVL5'-C	JO2
	164	MulgκVL5'-C	MulgλVL3'-1
V_H	γ72	V_H1BACK	(JO3 or JO4)
	1012	JO14 (1st PCR)	JO3
		JO14 (2nd PCR)	JO4
	1043	JO14 (1st PCR)	JO3
		(MulgVH5'-C + MulgVH5'-F) (2nd PCR)	MulgγH3'-2

Example 10: Isolation of Amplified BrE-3 V_L and V_H cDNA and Sequences

The PCR products were cloned without prior purification into pCR1000 (Invitrogen) and sequenced in both directions. Clones 152, 164, 1012, and 1043 were isolated independently during different RT-PCR runs. The sequences of V_L clones 152 and 164 were found to be identical, as were the sequences of the V_H clones 1012, 1043. The V_H and V_L DNA sequences and their derived protein sequences are shown in Tables 4 and 5 below.

Table 4: BrE-3 V_L Nucleotide and Derived Protein Sequences

BrE-3 V_L

DNA Sequence

ATG AAG TTG CCT GTT AGG CTG TTG GTG CTG TTG TTC TGG ATT CCT GCT TCC ATC AGT GAT GTT GTG ATG ACC CAA ACT CCA CTC TCC CTG CCT GTC AGT CTT GGA GAT CAA GCT TCC ATC TCT TGC AGA TCT AGT CAG AAC CTT GTA CAC AAC AAT GGA AAC ACC TAT TTA TAT TGG TTC CTG CAG AAG TCA GGC CAG TCT CCA AAG CTC CTG ATT TAT AGG GCT TCC ATC CGA TTT TCT GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCA GAG ACA GAT TTC ACA CTC AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TTC TGC TTT CAA GGT ACA CAT GTT CCG TGG ACG TTC GGT GGA GGC ACC AAG CTG GAA ATC AAA C (Seq. ID No: 10)

Amino Acid Sequence

m k l p v r l l v l L F W I P A S I S DI V V M T Q T P L S L P V S L G D Q A S I S C R S S Q N L V H N N G N T Y L Y W F L Q K S G Q S P K L L I Y R A S I R F S G V P D R F S G S G S E T D F T L K I S R V E A E D L G V Y F C F Q G T H V P W T F G G G T K L E I K (Seq. No: 11)

Table 5: BrE-3 V_H Nucleotide and Derived Protein Sequences

BrE-3 VH

DNA Sequence

ATG TAC TTG GGA CTG AAC TAT GTC TTC ATA GTT TTT CTC TTA AAA GGT GTC CAG AGT GAA GTG AAG CTT GAG GAG TCT GGA GGA GGC TTG GTG CAA CCT GGA GGA TCC ATG AAA CTC TCT TGT GCT GCT TCT GGA TTC ACT TTT AGT GAT GCC TGG ATG GAC TGG GTC CGC CAG TCT CCA GAG AAG GGG CTT GAG TGG GTT GCT GAA ATT AGA AAC AAA GCC AAT AAT CAT GCA ACA TAT TAT GAT GAG TCT GTG AAA GGG AGG TTCACC ATC TCA AGA GAT GAT TCC AAA AGT AGA GTG TAC CTG CAA ATG ATA AGC TTA AGA GCT GAA GAC ACT GGC CTT TAT TAC TGT ACT GGG GAG TTT GCT AAC TGG GGC CAG GGG ACT CTG GTC ACT GTC TCT GCA G (Seq. ID No: 12)

Amino Acid Sequence

m y l g l n y v f I V F L L K G V Q S EI V K L E E S G G G L V Q P G G S M K L S C A A S G F T F S D A W M D W V R Q S P E K G L E W V A E I R N K A N N H A T Y Y D E S V K G R F T I S R D D S K S R V Y L Q M I S L R A E D T G L Y Y C T G E F A N W G Q G T L V T V S A (Seq. ID No: 13)

The sequences were interpreted as described by Kabat et al. (1991). The residues that are shown in lower case correspond to PCR primers. The mature chains begin at D1 (V_L) and E1 (V_H), respectively. The amino-acids that are underlined are those corresponding to the CDRs. The underlined nucleotides indicate joining segments.

The framework and CDR polypeptide segments were identified according to Kabat et al. (1991). V_L is a group IIκ chain. Part of the CDR 3 and all of framework 4 (FR4) are encoded by J_{k1}. V_H belongs to group IIIc. CDR 3 and FR4 are encoded by J_{H3}. Little or nothing remains from an unidentified D minigene. Thus, the CDR 3 is only 4 amino-acids long.

Example 11: Comparison of cDNA deduced Amino Acid Sequence with Directly Determined N-Terminal Fragment Sequence

Table 6 below shows a comparison between the cDNA-derived polypeptide sequence and the polypeptide sequence determined directly from purified BrE-3 monoclonal antibody.

Table 6: Comparison of cDNA-Deduced Protein Sequence with Directly Determined N-terminal Protein Sequence

VL	cDNA-deduced	DVVMTQTPLSLPVSLGDQASISCRS
VL	Protein sequence	GVVMTQTPLSLPVVLGDQASIIXRX
VH	cDNA-deduced	EVKLEESGGGLVQPGGSMKLSCAAS
VH	Protein sequence	EVKLEESGGVLVQPGGSMKLSSAAS

BrE-3 was reduced with 5% mercaptoethanol, separated on a 10% SDS polyacrylamide gel, and electroblotted onto a ProBlott membrane (Applied Biosystems, Foster City, CA). Amino acid sequencing was performed directly on the immobilized bands by the Biotechnology Instrumentation Facility, University of California, Riverside. The protein sequence given here is the sequencer's best guess.

Once the variable region cDNAs were cloned, it was confirmed that, in fact, they encoded the variable regions of BrE-3 and not those of another antibody by comparing the cDNA-derived amino acid sequences of the cloned BrE-3 variable region with the N-terminal sequence of purified BrE-3 antibody directly determined by a single run of protein sequencing. The cDNA sequences were shown to be accurate by comparison with 2 independently reverse transcribed clones.

The general agreement between the predicted and the determined amino-acid sequences shows that the cloned cDNAs encode polypeptides of the same class and subclass as the variable regions of BrE-3. This indicates that the cDNAs encode authentic variable regions. The authenticity of the variable region polypetide and, therefore, that of the mouse-human chimeric BrE-3 antibody is unquestionable given that the variable regions and the chimeric antibody affinity constant is indistinguishable from that of BrE-3.

Example 12: **Construction of Mouse-Human Chimeric Antibody Genes**

The vectors used were developed by Coloma, M.J., et al. (Coloma, M.J., et al. (1992), supra) and kindly provided by S.L. Morrison (Dept. of Microbiology and Molecular Genetics, UCLA). Both vectors were derived from pSV2 (Mulligan, R.C., and Berg, P., "Expression of a Bacterial Gene in Mammalian Cells", Science 209:1422-1427 (1980)), and contain genomic fragments encoding either the heavy or the light chain constant domains. The vectors accept cDNAs that encode the F_v regions. To ligate the F_v cDNAs to the vectors, restriction ends were added to the cDNAs in a set of PCR reactions, using the JO16, JO17, JO18 and JO19 primers.

The pAG4622 light chain vector contains the gene for the human κ chain constant region, including the J-C intron. It encodes xanthine-guanine phosphoribosyltransferase or gpt (Mulligan, R.C., and Berg, P., "Selection for Animal Cells that Express the Escherichia Coli Gene Coding for Xanthine-Guanine Phosphoribosyltransferase", P.N.A.S. (USA) 78:2072-2076 (1981)) as a dominant selectable marker. It accepts the mouse VL cDNA between the ribosome binding site (Kozak, M., "Compilation and Analysis of Sequences

Upstream from the Translational Start Site in Eukaryotic mRNAs", Nucleic Acids Res. 12:857-872 (1984)), which is preceded by the VH promoter from the anti-dansyl murine monoclonal antibody 27.44 (Coloma, M.J., (1992), supra), and the J-C intron. The J-C intron contains the k chain enhancer (Potter, H., et al., "Enhancer-Dependent Expression of Human κ Immunoglobulin Genes Introduced into Mouse Prep-B Lymphocytes by Electroporation", P.N.A.S. (USA) 81:7161-7165 (1984); Emorine, L., et al., "A Conserved Sequence in the Immunoglobulin J Kappa-C Kappa Intron: Possible Enhancer Element", Nature 304: 447-449 (1983)).

The pAH4604 heavy chain vector contains the gene for the heavy chain γ1 constant region, but no J-C intron. It encodes histidinol-dehydrogenase or hisD (Hartman, S.C. and Mulligan, R.C. Two Dominant-Acting Selectable Markers for Gene Transfer Studies in Mammalian Cells", P.N.A.S. (USA) 85:8047-8051 (1988)) as a dominant selectable marker. It accepts the mouse V_H cDNA between the dansyl promoter-ribosome binding site and the constant γ1 gene. The vector also contains an insert that encodes the heavy chain enhancer (Rabbitts, T.H., et al, "Transcription Enhancer Identified Near the Human C mu Immunoglobulin Heavy Chain Gene is Unavailable to the Translocated c-myc Gene in a Burkitt Lymphoma", Nature 306:806-809 (1983)).

The new V_H and V_L DNA fragments with appropriate restriction ends were integrated into pAH4604 and pAG4622 as described in Example 4 above. The vectors were then electroporated (together) into SP2/0-Ag14 myeloma cells as described by Coloma et al. (1992), supra.

Example 13: Characterization of Mouse-Human Chimeric BrE-3 Antibody and V_H and V_L Regions

The supernatants from stable transfectants were assayed for the presence of the mouse-human chimeric antibody as described in Examples 6 and 7 above. High producing transfectants were subcloned and subsequently adapted to grow in serum-free medium. The mouse-human chimeric antibody produced by the myeloma cell line was then purified from the culture supernatant using a Sepharose 4B-protein A column (Bio-Rad, Richmond, CA) as described in Ey, P.L., et al. (Ey, P.L., et al., "Isolation of Pure IgG1, IgG2a and IgG2b Immunoglobulins from Mouse Serum Using Protein A-Sepharose", Immunochemistry 15:429-436 (1978)). Antibody disulfide bonds were reduced to separate the light and heavy chains by heating for 10 min at 65° in Laemmli loading buffer containing 5% beta-mercaptoethanol. The separated chains were then chromatographed on a SDS polyacrylamide gel (10%). The reduced mouse-human chimeric antibody and BrE-3 antibody were eletrophoresed in separate

lanes next to 97.4, 66.2, 45.0, 31.0 and 2.5 Kdalton protein markers. Table 7 below shows the apparent molecular weights of the two bands obtained for both.

Table 7: Reduced BrE-3 and Chimeric BrE-3 Antibody VL and VH Apparent Molecular Weights

Chimeric Antibody		BrE-3	
V_H (Kd)	V_L (Kd)	V_H (Kd)	V_H (Kd)
50	30	49	29

The heavy and light chains of the chimeric BrE-3 antibody separate as expected when electrophoresed on a polyacrylamide gel.

Example 14: Affinity Binding Constants for BrE-3 and Mouse-Human Chimeric Antibody

The purified mouse-human chimeric BrE-3 antibody and purified murine BrE-3 gave similar competition curves when tested against ^{125}I-labeled murine BrE-3 binding to its antigen. The affinity binding constants of the murine antibody and the mouse-human chimeric antibody were determined in independent competition assays as described in Example 8 above. The values of the constants are 2.68×10^8 M^{-1} and 3.75×10^8 M^{-1} for the hybrid BrE-3 polypeptides and for the murine antibody of BrE-3, respectively. These values are not distinguishable at a 95% confidence interval.

Example 15: Tissue Binding Studies

Immunohistochemical staining using the immunoperoxidase technique of consecutive human breast carcinoma tissue sections was conducted with the mouse-human chimeric BrE-3 antibody. A control was stained with the anti-human secondary antibody only. Positive staining resulted from the use of the mouse-human chimeric BrE-3 antibody, followed by the anti-human antibody specific binding. (Pictures not shown).

The breast carcinoma tissue sections were stained with the supernatant of the transfected cells using the Vectastain ABC method (Vector Labs, Burlingame, CA). The tissue stained with the goat anti-human Ig secondary antibody only shows background or non-specific staining of necrotic areas of the tissue section.

The tissue stained with mouse-human chimeric BrE-3 antibody, followed by the secondary antibody, shows specific staining of the breast carcinoma cells in the breast tissue sections.

Example 16: BrE-3 Imaging Studies

The murine monoclonal antibody BrE-3 has been shown to be highly effective for imaging and for the radioimmunotherapy of breast cancers. For example, in a pharmacokinetic study of 15 breast cancer patients conducted with an ^{111}In MXDTPA-BrE-3 radioimmunoconjugate (BrE-3 antibody), the serum levels were low in most patients, the blood clearance correlated with the circulating antigen and the imaging results showed that about 86% of all sites could be imaged (Liebes, L., et al., "Pharmacokinetics of ^{111}In-BrE-3 Monoclonal Antibody in Patients with Breast Carcinoma", Proc. Am. Assoc. Cancer Res. 33:216(Abs No. 1292) (1992)).

A ^{90}Y-BrE-3 radioimmunoconjugate having similar pharmacokinetic characteristics and extrapolating the ^{111}In-BrE-3 dosimetry results provide a superior therapeutic agent, as well.

As with many other monoclonal antibodies, however, the clinical applications of BrE-3, a whole mouse antibody, are limited by the HAMA response. A chimeric monoclonal antibody should give a more restricted HAMA response.

Example 17: Hybrid BrE-3 Immunogenicity

The BrE-3 variable region polypeptides have been cloned without the constant regions to produce less immunogenic polypeptides than the parent murine antibody. It has, moreover, been shown herein that the mouse-human chimeric BrE-3 antibody lacking its original murine constant region preserves its antigen binding characteristics.

A BrE-3 variable region chimeric alone or as a mouse-human chimeric antibody also containing a constant region human region or a fragment thereof is significantly less immunogenic to humans than the parent murine antibody. The hybrid polypeptide comprising the variable region of the BrE-3 antibody and the constant region of a human antibody was shown to preserve the original binding affinity of the murine antibody.

In this hybrid polypeptide, approximately 2/3 of its contiguous non-human immunogenic targets (C_L and C_H regions) were entirely replaced by human constant domains.

Example 18: Cloning of KC-4 V_H and V_L and cDNAs

The procedure and media employed are described and referenced in Examples 1and 2 above.

Example 19: PCR Primers used in First Isolation of KC-4 cDNAs

The PCR primers were purchased from Novagen (Madison, WI). Their sequences, reproduced from the booklet provided by Novagen, are shown in Table 8 below.

Table 8: PCR Primer Sequences

MulgκV_L5'-C: sense primer mix for kappa leader	
ACTAGTCGACATGAAGTTGCCTGTTAGGCTGTTGGTGCTG	(Seq. ID No: 14)
ACTAGTCGACATGGAGWCAGACACACTCCTGYTATGGGT	(Seq. ID No: 15)
ACTAGTCGACATGGATTTWCAGGTGCAGATTWTCAGCTTC	(Seq. ID No: 16)
MulgκV_L3'-1: antisense kappa constant region	
CCCAAGCTTACTGGATGGTGGGAAGATGGA	(Seq. ID No: 17)
MulgV_H5'-F: sense primer mix for heavy chain leader	
ACTAGTCGACATGRACTTTGGGYTCAGCTTGRTTT	(Seq. ID No: 18)
ACTAGTCGACATGAGAGTGCTGATTCTTTTGTG	(Seq. ID No: 19)
ACTAGTCGACATGGATTTTGGGCTGATTTTTTTTATTG	(Seq. ID No: 20)
MulgγV_H3'-2: antisense gamma constant region	
CCCAAGCTTCCAGGGRCCARKGGATARACIGRTGG	(Seq. ID No: 21)

Example 20: Cloning of Mouse-Human Chimeric KC-4 Antibody Ribonucleotide

The two expression vectors pAG4622 and pAH4604 described in Example 4 were utilized.

Oligonucleotides synthesized and used in a PCR to produce V_H and V_L fragments with the correct ends for insertion into the pAG4622 and pAH4604 expression vectors are shown in Table 9 below.

Table 9: PCR Primers Sequences

JO20 - sense kappa leader	
GGG GATATC CACC ATG AAG TTG CCT GTT AGG CTG TTG	(Seq. ID No: 22)
JO21 - antisense JK2	
CCC GTCGACTTAC G TTT TAT TTC CAG CTT GGT CCC CCC T	(Seq. ID No: 23)
JO22 - sense V_H leader	
GGG GATATC CACC ATG GAC TTT GGG CTC AGC TTG GTT TT	(Seq. ID No: 24)
JO24 - antisense JH3	
CCC GCTAGC TGC AGA GAC AGA GAC CAG AGT CC	(Seq. ID No: 25)

The original pCR1000 clones were the starting templates for the PCR and the rest of the procedures as described in Example 4 above.

Example 21: Expression of the anti-KC-4 Chimeric Gene

The V_H and V_L regions in the KC-4 mouse-human chimeric antibody were sequenced once again to verify that their sequences were correct. The transfection of the non-producer myeloma cell line SP2/0-Ag14, (ATCC: CRL 1581) and isolation of polypeptide was conducted as described in Example 5 above.

Example 22: Production of Transfected Hosts

After ten days, stable transfectant colonies were clearly established at a frequency of approximately 1/10,000. The colonies were transferred to normal medium and the assays conducted as described in Example 6 above.

Example 23: Confirmation of Mouse-Human Chimeric KC-4 Antibody Expression

The supernatants were essayed for binding to human milk fat globule (HMFG) and the breast epithelial mucin (BEM) as described previously in Example 7 above. HMFG and BEM were bound to the microtiter plates as described previously by Ceriani, R.L. (1990). In this radioassay the bound chimeric KC-4 (HMFG and BEM) was detected by anti-human gamma chain conjugated to ^{125}I. Most colony supernatants were positive by both assays. The colonies that secreted the highest level of chimeric antibody in the supernatants, as determined by these assays, were subcloned.

Example 24: Western Blot

75 µl of the culture supernatant was added to 20 µl of 4x Laemmli buffer and 5 µl β-mercaptoethanol and the mixture was heated at 65 °C for 15 min., in order to reduce antibody disulfide bonds and, thus, separate heavy from light chains. 20 µl of the treated sample was chromatographed in duplicate lanes on a 10% SDS polyacrylamide gel together with other antibodies that were treated similarly and that were loaded for comparison. Pre-stained size markers (BioRad, Richmond, CA) were also loaded.

The chromatographed proteins were electroblotted onto a ProBlott membrane (Applied Biosystems, Foster City, CA) in 90% 30 mM CAPS pH11, 10% methanol, for 1 hour at 25 V and at 4 °C. The membrane was cut into 2 parts containing identical antibody samples. The 2 membranes were immersed in 20% Bovine Calf Serum in PBS

and shaken slowly at room temperature for 1hour 35 min. ^{125}I labeled goat anti-human κ chain antibody was added to one membrane and ^{125}I labeled goat anti-human γ chain antibody to the other membrane. Antibodies were labeled at a specific activity of approximately 10 mCi/mg using the chloramine T method as described by Ceriani, R.L. and Blank, E.W. (1988), the labeled antibodies were diluted to 4,000 cpm/μl in RIA buffer.

After incubating 3 hours at room temperature the blots were washed twice in TBS for 10 min each time, once in TBST (50 mM TRIS pH7.5, 3 mM EDTA 25 mM NaCl) 10 min and once more in TBS (TBS with 0.05% Tween 20) for 10 min. The membranes were dried and exposed to Kodak XAR film.

Western blot analyses of culture supernatants revealed that 3 antibody chains were expressed that corresponded to the 3 antibody chains seen in the original KC-4 antibody. These were a heavy chain that stained with goat anti-human γ chain ^{125}I-labeled antibody, and 2 light chains that stained with goat anti-human κ chain ^{125}I-labeled antibody (Figure not shown).

The treatment of the original murine KC-4 antibody with N-glycosidase F (Boehringer Mannheim GmbH Germany) following the recommendations of the manufacturer, produced a noticeable decrease in the intensity of the "top" light chain and a concomitant increase in the intensity of the bottom light chain (Figure not shown).

The explanation for the existence of an extra light chain is that this chain is glycosylated. Three lines of evidence substantiate this. First, the detection of an asparagine-linked glycosylation site in the amino acid sequence of the light chain. That is the triad NIS (Asn-Ile-Ser) in framework 3. Second, the decrease of the intensity in the putative glycosylated band after treatment with N-glycosidase F, while concomitantly the intensity of the non-glycosylated band was increased. Finally, 2 corresponding light chain bands are seen in the chimeric antibody version.

The extra light chain in the chimeric version cannot be a contaminant since it was specifically stained by goat anti-human κ chain antibody. It can only be a product expressed by pAG4622. Thus both light chains must have the same V_L amino acid sequence and the same human constant region. These observations show that approximately half of the light chains of both the murine KC-4 and the KC-4 chimeric antibody are glycosylated at the asparagine-linked glycosylation site.

Example 25: Amplification of cDNAs Encoding KC-4 F_v Regions

The cDNAs that encode the KC-4 mouse immunoglobulin V_H and V_L were prepared as described in Example 9 above from polyadenylated RNA isolated

from 100 million KC-4 hybridoma cells. All clones were obtained from independent PCRs. The sequences of the primers are given in Example 19 and 20 above. All primers are specific for either the leader peptide region or for the constant regions. The primer combinations utilized herein are shown in Table 10 below.

Table 10: Primer Combination for PCR Amplifications

Clone No.	Primer combinations	
V_L	96	MulgκV_L5'-C + MulgκV_L3'-1
	107	MulgκV_L5'-C + MulgκV_L3'-1
	K1	JO20 + JO21
V_H	66	MulgV_H5'-F + MulgγV_H3'-2
	209	MulgV_H5'-F + MulgγV_H3'-2
	H3	JO22 + JO24
	H7	JO22 + JO24

Example 26: Isolation of Amplified KC-4 F_{VL} (V_L) and F_{VH} (V_H) cDNA and sequences

The PCR products were cloned, without prior purification, into pCR1000 (Invitrogen) and sequenced in both directions. The V_H and V_L DNA sequences and their derived protein sequences are shown in Tables 11 and 12 below.

Table 11: V_L Nucleotide Sequences

KC-4 V_L (kII-Jk2)

ATG AAG TTG CCT GTT AGG CTG TTG GTG CTG ATG TTC TGG ATT CCT
GCT TCC AGC AGT GAT GTT TTG ATG ACC CAA ACT CCT CTC TCC CTG
CCT GTC AGT CTT GGA GAT CAA GCC TCC ATC TCT TGC AGA TCT AGT
CAG AGC ATT GTA CAT AGT AAT GGA AAC ACC TAT TTA GAA TGG TAC
CTG CAG AAA CCA GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT
TCC ATC CGA TTT TCT GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA
TCA GGG ACA GAT TTC ACA CTC AAT ATC AGC AGA GTG GAG GCT GAG
GAT CTG GGA ATT TAT TAC TGC TTT CAA GGT TCA CAT GTT CCG TAC
ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA C (Seq. ID No: 26)

Table 12: V_H Nucleotide Sequence

KC-4 V_H (IIID-D9-JH3)

ATG GAC TTT GGG CTC AGC TTG GTT TTC CTT GTC CTT ATT TTA AAA GGT GTC CAG TGT
GAA GTG CAG ATG GTG GAG TCT GGG GGA GGC TTA GTG AAG CCT GGA GGG TCC CTG AAA
CTC TCC TGT GCA GCC TCT GGA TTC GCT TTC AGT AGC TAT GCC ATG TCT TGG GTT CGC
CAG TCT CCA GAG AAG AGG CTG GAG TGG GTC GCA GAA ATT AGT AGT GGT GGT AAT TAC
GCC TAC TAT CAA GAC ACT GTG ACG GGC CGA TTC ACC ATC TCC AGA GAC AAT GCC AAG
AAC ACC CTG TAC CTG GAA ATG AGC AGT CTG AGG TCT GAG GAC ACG GCC ATG TAT TAC
TGT GCA AGG GAG GAC TAC GGT ATC CCG GCC TGG TTT GCT TAC TGG GGC CAA GGG ACT
CTG GTC TCT GTC TCT GCA G (Seq. ID No: 27)

Example 27: Amino Acid Sequences of KC-4 Chimeric Antibody Fv Regions

After the KC-4 F_v region cDNAS were cloned, and sequenced, and their cDNA-derived amino acid sequence was compared with the N-terminus sequence directly determined by a single run of amino acid sequencing on purified KC-4 antibody.

The cDNA sequences were shown to be accurate since in both cases they were identical for clones that were prepared from independent reverse transcription reactions. This confirms that the cloned cDNAs are authentic KC-4 F_v regions. The sequences are shown in Tables 13 and 14 below.

Table 13: VL Amino Acid Sequences

	KC-4V_L(kII-Jk2)
	MKLPVRLLVLMFWIPASSS (Seq. ID No: 28)
FR1	DVLMTQTPLSLPVSLGDQASISC (Seq. ID No: 29)
CDR1	RSSQSIVHSNGNTYLE (Seq. ID No: 30)
FR2	WYLQKPGQSPKLLIY (Seq. ID No: 31)
CDR2	KVSIRFS (Seq. ID No: 32)
FR3	GVPDRFSGSGSGTDFTLNISRVEAEDLGIYYC (Seq. ID No: 33)
CDR3	FQGSHVPYT (Seq. ID No:34)
FR4	FGGGTKLEIK (Seq. ID No. 35)

Table 14: V_H Amino Acid Sequences

	KC-4V_H (IIID-D9-JH3)
	MDFGLSLVFLVLILKGVQC (Seq. ID No: 36)
FR1	EVQMVESGGGLVKPGGSLKLSCAASGFAFS (Seq. ID No:37)
CDR1	SYAMS (Seq. ID No: 38)
FR2	WVRQSPEKRLEWVA (Seq. ID No: 39)
CDR2	EISSGGNYAYYQDTVTG (Seq. ID No: 40)
FR3	RFTISRDNAKNTLYLEMSSLRSEDTAMYYCAR (Seq. ID No: 41)
CDR3	EDYGIPAWFAY (Seq. ID No: 42)
FR4	WGQGTLVSVSA (Seq. ID No: 43)

The sequences were interpreted as described by Kabat et al. (1991), supra. The residues that are underlined correspond to PCR primers. The mature V_L and V_H chains begin at amino-acids D and E of framework 1 (FR1), respectively.

Framework and CDR protein segments were identified according to Kabat et al. (1991), supra. V_L is a group II κ chain. Part of the CDR 3 and all of the framework 4 (FR4) are encoded by Jk2. V_H belongs to group IIId. CDR 3 and FR4 resulted from a genomic recombination involving minigenes D9 and JH3. There is an asparagine glycosylation site in the light chain in FR3. The site reads NIS (Asn Ile Ser).

Example 28: Comparison of cDNA deduced Amino Acid Sequence with Directly Determined N-Terminal Fragment sequence

A comparison between the cDNA-derived polypeptide sequence and the amino acid sequence determined directly on the purified KC-4 monoclonal antibody was undertaken. The results are shown in Table 15 below.

Table 15: Comparison of cDNA-deduced with Direcly Determined N-Terminal Amino Acid Sequences

	FIRST BAND TOP	
V_H,	cDNA-deduced	EVQMVESGGGLVKPGGSLKLS (Seq. ID No: 44
V_H,	Protein sequence	EVQMVESGGGLVKPGGXLKLS (Seq. ID No: 45)
	SECOND BAND	
V_L,	cDNA-deduced	DVLMTQTPLSLPVSLGDQASI (Seq. ID No: 46)
V_L,	Protein sequence	DVLMTQTPLSLPVXXGDQASI (Seq. ID No: 47)
	THIRD BAND	
V_L,	cDNA-deduced	DVLMTQTPLSLPVSLGDQASI (Seq. ID No: 48)
V_L,	Protein sequence	DVLMTQTPLSLPVSLGDQASI (Seq. ID No: 49)
X	uncertain or alternative calls.	

A sample of KC-4 (approximatly 190 ug) was reduced with 5% mercaptoethanol (65°C for 15 min.), separated on three lanes of a 10% SDS polyacrylamide gel, and electroblotted onto a ProBlott membrane (Applied Biosystems, Foster City, CA) in 90% 30 mM CAPS pH11, 10% methanol, for 1 hour at 25 V and at 4°C. The transferred protein species were stained with Commassie Blue. 3 bands were seen in each lane, of which 2 migrated as expected for a heavy and light chain. The third band migrated above the light chain. Amino acid sequencing was performed directly on the immobilized bands by the Biotechnology Instrumentation Facility, University of California, Riverside. The amino acid sequence given here is the sequencer's best guess.

Example 29: Construction of Mouse-Human Chimeric KC-4 Antibody Genes

The vector used were described in Example 1 above. Restriction ends were added to the cDNAs in a set of PCR reactions, using primers JO20, 21, 22, and 24.

The pAG4622 light chain vector and the pAH4604 heavy chain vector were described in Example 12 above.

The new V_H and V_L DNA fragments with appropriate restriction ends were integrated into pAH4604 and pAG4622 as described in Example 12 above. The vectors were then electroporated (together) also as described in Example 12.

Example 30: Tissue Binding Studies

The supernatants from stable transfectants were assayed for the presence of the mouse-human chimeric KC-4 antibody as described in Example 13. The chimeric antibody secreted in the supernatant bound both HMFG and BEM very strongly. In addition, the supernatants containing mouse-human chimeric KC-4 antibody were used to stain human breast carcinoma tissue sections by using the immunoperoxidase immunohistochemical staining technique. The intensity of the staining was comparable to that obtained with the original murine monoclonal antibody. The KC-4 monoclonal antibody is known to bind the human milk fat globule and the breast epithelial mucin. This binding specificity of the KC-4 monoclonal antibody was maintained after the recombinant procedure. The KC-4 chimeric antibody bound very strongly to the human milk fat globule and the breast epithelial mucin as determined by radioassay (Ceriani, et al., Breast Cancer Res. Trent. 15:161 (1990)). In addition, the KC-4 chimeric antibody bound several human breast tumors in histopathological sections in a manner comparable to the KC-4 murine monoclonal antibody, as detected by immunostaining described in Example 15 above. This specificity of binding demonstrated the retained binding reactivity of the variable regions of KC-4 by the polypeptide of the invention when attached to the human F_c fragment.

Example 31: Hybridoma Cell Deposits

The hybridoma cell lines expressing the BrE-3 and anti-KC-4 mouse-human chimeric antibodies were deposited on November 13, 1992 under the Budapest Treaty with the ATCC and have been assigned Accession Nos. HB 11199 (Chimeric BrE-3 A1C10) and HB 11201 (Chimeric KC-4 1E8).

Example 32: Materials and Assays for Epitope Mapping

The specific details of the preparation of materials, cell lines, and techniques employed were disclosed by Peterson, J.A., et al. (Peterson, J.A., et al., "Molecular Analysis of Epitope Heterogeneity of the Breast Mucin", Breast Epithelial Antigens, Ed. Ceriani, R.L., Plenum Press, NY (1991)), the relevant text

of which is incorporated herein by reference. Overlapping peptide hexamers were synthesized onto the ends of polyethylene pins using an Epitope Scanning Kit (Cambridge Research Biochemicals, Cambridge, UK), which is based on a method originally described by Geysen, H.L., et al. (Geysen, H.L., et al., "Use of Peptide Synthesis to Probe Vital Antigens for Epitopes to a Resolution of a Single Amino Acid", P.N.A.S. (USA) 81:3998-4002 (1984)). The polyethylene pins were arranged in a 8x12 configuration that fits into a 96 well microtiter dish. The pins are supplied with an alanine attached to the ends to which the amino acids are added consecutively using pentafluorophenyl active esters of fluorenylmethyloxycarbonyl (Fmoc)-L-amino acids. Each consecutive overlapping hexamer differs from the previous one by a single amino acid and enough were synthesized to span the entire sequence of the peptide to be tested so that every combination of hexamer was present. Each monoclonal antibody was tested for binding to the synthetic peptides using an ELISA method with horse radish peroxidase-conjugated goat anti-mouse IgG (Promega, Madison, WI) and color development with 2,2'azinobis (3-ethylbenzothiazoline- 6-sulfonic acid (Sigma, St. Louis, MO). The hexapeptides starting with A,P, D, and T bind well to the antibodies (Hexamers 1 to 3 and 20), whereas the hexamers starting between these positions did not. The hexamers prepared are shown in Table 16 below. From the hexamer that each monoclonal antibody binds the linear amino acid sequence essential for its binding to the antigen may be deduced. For example, BrE-3 required the sequence TRP within the hexamer. Other monoclonal antibodies required other amino acid sequences (e.g., Mc5, TRPAP; Mc1, DTR; BrE-1, DTRP). BrE-2 also required TRP but its different specificity for normal and tumor tissue indicates that its epitope on the native antigen is different from BrE-3.

Table 16: Epitope Mapping of Repeat Peptide Breast Mucin

Hexamer	G V T S A P D T R P A P G S T A P P A H G V T S A P D T R P
1	P D T R P A
2	D T R P A P
3	T R P A P G
4	R P A P G S
5	P A P G S T
6	A P G S T A
7	P G S T A P
8	G S T A P P
9	S T A P P A
10	T A P P A H
11	A P P A H G
12	P P A H G V
13	P A H G V T
14	A H G V T S
15	H G V T S A
16	G V T S A P
17	V T S A P D
18	T S A P D T
19	S A P D T R
20	A P D T R P

Example 33: Epitope Mapping

Five different monoclonal antibodies (Mc1, Mc5, BrE1, BrE2 and BrE3), were prepared using the human milk fat globule (HMFG) for immunization. All identified epitopes on the highly glycosylated large molecular weight breast mucin. By immunohistochemistry they appeared to recognize different epitopes since each had different tissue and tumor specificities (Peterson, J.A., et al., "Biochemical and Histological Characterization of Antigens Preferentially Expressed on the Surface and Cytoplasm of Breast Carcinomas Cells Identified by Monoclonal Antibodies Against the Human Milk Fat Globule", Hybridoma 9:221-235 (1990)). Each monoclonal antibody bound to a different spectrum of normal tissues and their specificities for different carcinomas were different. BrE2 and BrE3, however, were quite similar. In addition, by screening breast *ygtll* cDNA expression libraries with some of these monoclonal antibodies, cDNA clones were isolated that produced fusion proteins that bound all of them, while other cDNA clones bound just some (Larroca, D., et al., "High Level Expression in E. Coli of an Alternate Reading Frame of pS2 mRNA that Encodes a Mimotope of Human Breast Epithelial Mucin Tandem Repeat" Hybridoma 11(2):191-201 (1992)).

This binding to the fusion proteins indicated that the epitopes for these 5 monoclonal antibodies included the polypeptide portion of this glycoprotein. To confirm this the binding of these monoclonal antibodies to two synthetic polypeptide 20-mers (PDTRPAPGSTAPPAHGVTSA and APPAHGVTSAPDTRPAPGST) that spanned the tandem repeat consensus sequence was tested (Gendler, S.J., et al., "Cloning of Partial cDNA Encoding

Differentiation and Tumor-Associated Mucin Glycoproteins Expressed by Human Mammary Epithelium", P.N.A.S. (USA) 84:6060-6064 (1987); Siddiqui, J., et al., "Isolation and Sequencing of a cDNA Coding for the HUman DF3 Breast Carcinoma-Associated Antigen", P.N.A.S. (USA) 85:2320-2323 (1988)).

One was started at the beginning of the published 20 amino acid repeat (Gendler, S.J., et al. (1987), supra) unit, and the other was started in the middle. All five monoclonal antibodies bound to both synthetic peptides, as did DF3, a monoclonal antibody against breast carcinoma cells produced by others (Hull, S.R., et al., "Oligosaccharide Diffeences in the DF3 Sialomucin Antigen from Normal Human Milk and the BT-20 Human Breast Carcinoma Cell Line", Cancer Comm. 1:261-267 (1989)). Three other monoclonal antibodies (Ceriani, R.L., et al., "Characterization of Cell Surface Antigens of Human Mammary Epithelial Cells with Monoclonal Antibodies Prepared Against Human Milk Fat Globule", Somat. Cell Genet. 9:415-427 (1982); Peterson, J.A., et al., "Biochemical and Histological Characterization of Antigens Preferentially Expressed on the Surface and Cytoplasm of Breast Carcinoma Cells Identified by Monoclonal Antibodies Against the Human Milk Fat Globule", Hybridoma 9:221-235 (1990)) against other components of the HMFG that do not cross-react with the breast mucin, Mc13, against a 70 KDa glycoprotein, and Mc3 and Mc8, against a 46 KDa glycoprotein do not bind to these synthetic peptides (data not shown).

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the invention as set forth herein.

WHAT IS CLAIMED AS NOVEL AND UNOBVIOUS AND DESIRED TO BE PATENTED IN LETTERS PATENT IS:

1. A pure, isolated polypeptide which selectively binds to an antigen present on the surface or in the cytoplasm of neoplastic cells or that is released by the cells, the polypeptide being selected from the group consisting essentially of at least one variable region of the light or heavy chains of an antibody of a first species having affinity and specificity for the human milk fat globule (HMFG) and for an antigen found on the surface or the cytoplasm of a tumor cell or that released by the cell; combinations thereof, wherein each polypeptide is operatively linked to at least one other polypeptide; and mixtures thereof.

2. The anti-tumor polypetide of claim 1, wherein the antibody is selected from the group consisting of mouse, rat, goat, rabbit, primate, horse, bovine, ovine, pig, and guinea pig antibodies.

3. The anti-tumor polypetide of claim 1, having an amino acid sequence selected from the group consisting of amino acid Sequence ID No: 11 starting at amino acid D1; amino acid Sequence ID No: 13 starting at amino acid E1; amino acid Sequence ID No: 29 to 35; amino acid sequence ID No.: 37 to 43; combinations thereof wherein each polypeptide is operatively linked to at least one other polypeptide; and mixtures thereof.

4. A glycosylated polypeptide which selectively binds to an antigen present on the surface or in the cytoplasm of neoplastic cells or that is released by the cells, comprising the polypeptide of claim 1; and at least one glycosyl residue operatively linked to the polypeptide.

5. A composition of matter, comprising the polypeptide of claim 1; and a pharmaceutically-acceptable carrier.

6. A diagnostic kit for tumor neoplasias, comprising the composition of claim 5; a solid support; anti-tumor antibody; anti-constant region immunoglobulin, protein G or protein A; and instructions for its use.

7. An anti-tumor hybrid polypeptide, comprising at least one anti-tumor polypeptide of claim 1 and at least one effector agent operatively linked to the polypeptide; combinations thereof; and mixtures thereof.

8. The anti-tumor hybrid polypeptide of claim 7, wherein the effector agent is selected from the group consisting of non-peptide polymers, monomers and peptide polymers other than the constant region of an antibody of the same species.

9. The anti-tumor hybrid polypeptide of claim 8, wherein the effector agent comprises a radioisotope, an enzyme or a fluorescent label.

10. The anti-tumor hybrid polypeptide of claim 8, wherein the effector agent comprises a monomer selected from the group consisting of therapeutic,

immunogenic and diagnostic agents, radioisotopes, DNA monomers, RNA monomers, chemical linkers, and transmitter molecules, combinations thereof, and combinations thereof with peptide and non-peptide polymers or copolymers.

11. The anti-tumor hybrid polypeptide of claim 8, wherein the effector agent comprising a non-peptide polymer is selected from the group consisting of ester, ether, vinyl, amido, imido, alkylene, arylalkylene, cyanate, urethane, and isoprene polymers, halogenated polymers, DNA polymers, RNA polymers,copolymers thereof, and copolymers thereof with peptide polymers or monomers.

12. The anti-tumor hybrid polypeptide of claim 8, wherein the effector agent comprising a peptide polymer is selected from the group consisting of the constant region of human antibodies or fragments thereof, variable regions of antibodies, hormones, enzymes, toxins, complement, peptide transmitters, whole antibodies, Fab , Fab' and $(Fab')_2$ antibody fragments, combinations thereof, and combination thereof with non-peptide polymers, copolymers or monomers.

13. The anti-tumor hybrid polypeptide of claim 12, wherein the polypeptide comprises non-human amino acid sequences; and the peptide polymer comprises at least one constant region of the light or heavy chains of a human antibody or fragments thereof capable of binding to anti-constant region immunoglobulin, protein G or protein A.

14. The anti-tumor hybrid polypeptide of claim 13, being selected from the group consisting of a chimeric polypeptide comprising two heavy and two light chains, each light and heavy chain comprising at least one non-human variable region polypeptide and at least one constant region or fragment thereof of a human antibody; at least one chimeric Fab, Fab' or $(Fab')_2$ fragment thereof; combinations thereof; and mixtures thereof.

15. The anti-tumor hybrid polypeptide of claim 14, wherein each pair of heavy and light chains has different specificity.

16. The anti-tumor hybrid polypeptide of claim 8, wherein the polypeptide and the effector agent are operatively linked by a polymer.

17. The anti-tumor hybrid polypeptide of claim 7, being selected from the group consisting of at least one variable region of the heavy chain of a first antibody and a first effector agent; at least one variable region of the light chain of a second antibody and a second effector agent, wherein each pair of light and heavy chains has a predetermined specificity; combinations thereof; and mixtures thereof.

18. The anti-tumor hybrid polypeptide of claim 7, wherein at least one variable region of the heavy chain of the non-human antibody and at least one variable region of the light chain of the non-human antibody are linked to one

another.

19. The anti-tumor hybrid polypeptide of claim 7, wherein at least one of a first pair of light and heavy chains comprising at least one variable region is linked to at least one of a second pair of light and heavy chains comprising at least one variable region.

20. An anti-tumor composition, comprising the anti-tumor hybrid polypeptide of claim 7; and a pharmaceutically-acceptable carrier.

21. A diagnostic kit for neoplasms, comprising the anti-tumor composition of claim 20, wherein the effector agent is a constant region of an antibody or a fragment thereof that binds anti-constant region immunoglobulin, protein G or protein A; anti-constant region immunoglobulin, protein G or protein A; a solid support having operatively linked thereto an antigen which selectively binds to the anti-tumor hybrid polypeptide and the antibody; and instructions for its use.

22. An in vivo method of imaging and/or diagnosing a neoplasia, comprising administering to a subject suspected of being afflicted with a primary or metastasized neoplasia, the anti-tumor hybrid polypeptide of claim 9 in radiolabeled form, in an amount effective to reach the neoplasia and bind thereto; and detecting any localized binding of the labeled hybrid polypeptide to the tumor.

23. The in vivo method of imaging and/or diagnosing a neoplasia of claim 22, wherein the anti-tumor hybrid polypeptide is administered in an amount of about 0.001 to 100 μg/kg weight per treatment.

24. An in vitro method of diagnosing a neoplasia, comprising obtaining a biological sample from a subject suspected of being afflicted with a neoplasia; contacting the sample with the anti-tumor polypeptide of claim 1 to form an anti-tumor polypeptide-antigen complex with any neoplastic antigen present in the sample; and detecting any complex formed.

25. The in vitro method of diagnosing a neoplasia of claim 24, wherein the anti-tumor polypeptide added to the biological sample comprises a labeled polypeptide.

26. An in vitro method of diagnosing a neoplasia, comprising contacting a biological sample with a known amount of the polypeptide of claim 12, in the presence of an antigen molecule that specifically binds to the polypeptide, the antigen molecule being operatively linked to a solid support to form a polypeptide-antigen molecule complex on the solid support and a polypeptide-sample antigen complex with any neoplastic cell antigen present in the sample; detecting any complex formed between the polypeptide and the solid supported neoplastic antigen; and comparing the result with a control conducted in the

absence of the sample.

27. A method of inhibiting the growth or reducing the size of a primary or metastasized neoplastic tumor comprising administering to a human in need of the treatment an effective amount of the anti-tumor hybrid polypeptide of claim 9.

28. The method of inhibiting the growth or reducing the size of a neoplastic tumor of claim 27, wherein the hybrid polypeptide is administered in an amount of about 0.001 to 200 μg/kg body weight per dose.

29. A composition comprising an oligodeoxyribonucleotide encoding the polypeptide of claim 1.

30. The composition of claim 29, wherein the oligodeoxyribonucleotide comprises a DNA sequence selected from the group consisting of DNA Sequence ID No: 10 starting at codon 20 (GAT); DNA Sequence ID No: 12 starting at codon 20 (GAA); DNA Sequence ID No: 26, codons 20 to the last codon; DNA Sequence ID No: 27, codons 20 to the last codon; redundant DNA sequences thereof; combinations thereof; and mixtures thereof.

31. A composition comprising a vector carrying the polydeoxyribonucleotide of claim 30 operatively linked thereto.

32. The composition of claim 31, wherein the hybrid vector further comprises an oligodeoxyribonucleotide encoding an effector polypeptide, the effector polypeptide-encoding polydeoxyribonucleotide being operatively linked to the vector.

33. The composition of claim 32, wherein the oligodeoxyribonucleotide encodes a polypeptide selected from the group consisting of at least one variable region of the heavy and the light chains of the antibody and combinations thereof; and the effector peptide-encoding oligodeoxyribonucleotide is operatively linked to the oligodeoxyribonucleotide so as to express a hybrid polypeptide comprising at least one variable region of the light or heavy chains of the polypeptide or combinations thereof, and the effector peptide.

34. A composition comprising a host cell transfected with the hybrid vector of claim 31.

35. The transfected cell of claim 34, wherein the hybrid polypeptide comprises an antibody formed of a murine variable region and a human constant region.

36. A transfected host cell having the ATCC Accession Nos.11200 or HB 11201.

37. A composition comprising an oligoribonucleotide encoding the anti-tumor polypeptide of claim 1.

38. A method of producing a polypeptide which specifically binds to an

antigen present on the surface or in the cytoplasm of neoplastic cells, or that is released by the cells, comprising a) cloning the polydeoxyribonucleotide of claim 29 into a vector to form a hybrid vector; b) transfecting a host cell with the hybrid vector and allowing the expression of the anti-tumor polypeptide; and c) isolating the anti-tumor polypeptide or mixtures thereof.

39. The method of claim 38, wherein steps a) and b) are conducted by cloning polydeoxyribonucleotides encoding polypeptides selected from the group consisiting of at least one variable region of the heavy or light chains of the non-human antibody; and the method further comprises d) allowing the expressed polypeptides to interact with one another to form double chain polypeptides.

40. A method of producing an anti-tumor hybrid polypeptide comprising a polypeptide which selectively binds to an antigen present on the surface or in the cytoplasm of neoplastic cells or to an antigen that is released by the cells and an effector peptide, the method comprising a) transfecting a host cell with the hybrid vector of claim 32 and allowing the expression of the anti-tumor hybrid polypeptide; and b) isolating the anti-tumor hybrid polypeptide or mixtures thereof.

41. An ex vivo method of purging neoplastic cells from a biological fluid, comprising obtaining a biological fluid from a subject suspected of being afflicted with a neoplasia; contacting the fluid with an amount of the polypeptide of claim 12 effective to bind neoplastic cells present in the fluid; inactivating the cells; and replenishing the fluid to the subject.

42. The method of claim 41, wherein the inactivation of the neoplastic cells is attained by adding a cell inactivating agent that is active on the cells when bound to the polypeptide or by separating any polypeptide-neoplastic cell complexes formed from the remainder of the fluid.

43. An in vitro histochemical method of assessing the presence of neoplastic cells in a tissue, comprising obtaining a tissue sample from a patient suspected of being afflicted with a neoplasia and preparing a tissue substrate therefrom; contacting the tissue substrate with the polypeptide of claim 9 and allowing the polypeptide to bind to neoplastic cells present therein; and detecting the presence of any complexes formed.

44. A composition comprising an anti-idiotype polypeptide, comprising polyclonal antibodies raised against the polypeptide of claim 1; monoclonal antibodies thereof capable of specifically binding the anti-tumor antibody; fragments thereof selected from the group consisting of Fab, Fab', $(Fab')_2$ and variable region fragments thereof; an oligopeptide comprising the amino acid sequence APDTRPA or tandem repeats thereof; combinations thereof, wherein each oligopeptide, antibody or fragment thereof is operatively linked to at least

one other oligopeptide antibody or fragment thereof; or mixtures thereof.

45. The composition of claim 44, wherein the anti-idiotype polypeptide selectively binds to at least one variable region of the light or heavy chains of the polypeptide.

46. The composition of claim 44, wherein the anti-idiotype polypeptide, further comprises an effector agent operatively linked thereto.

47. An anti-neoplastic vaccine, comprising the anti-idiotype polypeptide of claim 44; and a pharmaceutically-acceptable carrier.

48. The anti-neoplastic vaccine of claim 47, in unit form.

49. An anti-tumor vaccination kit, comprising the vaccine of claim 46 and a diluent, in separate sterile containers; and instructions for its use.

50. A method of vaccinating against neoplastic tumors, comprising administering to a mammalian subject an effective amount of the anti-idiotype polypeptide of claim 44.

51. The vaccination method of claim 50, wherein the anti-idiotype polypeptide is administered in an amount of about 0.1 to 500 μg/kg body weight/dose.

52. A method of lowering the serum concentration of a circulating polypeptide that binds to an antigen present on the surface or in the cytoplasm of neoplastic cells or that is released by the cells, comprising administering to a human the anti-idiotype polypeptide of claim 44 in an amount effective to bind the circulating polypeptide to thereby accelerate its clearance.

53. The method of claim 42, wherein the binding polypeptide is administered in an amount of about 0.01 to 100.00 μg/kg body weight/dose.

54. A method of inhibiting the growth or reducing the size of a primary or metastasized neoplastic tumor comprising administering to a mammalian subject in need of the treatment an effective amount of an anti-tumor hybrid polypeptide comprising an effector agent selected from the group consist of radioisotopes, therapeutic drugs and vaccines, and an anti-tumor polypeptide which selectively binds to the HMFG antigen and to an antigen present on the surface or in the cytoplasm of human carcinoma cells or that is released by the cells; allowing the hybrid polypeptide to reach the tumor and the polypeptide to bind thereto; and administering to the human an amount of the anti-idiotype polypeptide of claim 44 effective to bind any residual or unbound circulating hybrid polypeptide to thereby accelerate the clearance of the hybrid polypeptide.